

New government, old problems

The surprise re-election of the Conservative British government may not be as bad for British science as the past decade would suggest. Much will depend on new people's willingness to listen to the truth.

AGAINST the bookmakers' odds and the confident predictions of the commercial polling organizations, the British government was re-elected last week. Although its majority is small, it is enough to keep it in office for a full five years. On the face of things, that is bad news for the British research enterprise. Why should a government convinced that it has done as well by science as anybody could have expected now change its view, especially after an electoral upset?

Luckily, there is some hope. The Prime Minister, Mr John Major, has been speaking of a government "of all the people"; the hope must be that even researchers and academics are counted among them. Then, as is customary, there is a change of faces at the two ministries with an important influence on research. Mainland-minded Mr Michael Heseltine, with hankering after industrial policy (or public support for chosen innovation), will run the Department of Trade and Industry (DTI). And Mr John Patten, previously at the Home Office, becomes the sixth Secretary of State for Education and Science in twelve years; the hope is that, with a Cambridge PhD and a spell as an Oxford academic, he will at least acknowledge that there is a problem to be tackled. If he plays his cards well, he may yet collect the honorary degree Oxford's academics denied to Mrs Margaret Thatcher a decade ago. Certainly, neither of the new men needs feel bound by the complacency of his predecessor.

The diagnosis is now well-known: British researchers are mostly demoralized, underpaid and inadequately supported in research, while the institutions in which they work (universities and public research organizations) are so much in flux that opportunities for long-term programmes of research have dwindled. Demoralization, being a state of mind, cannot confidently be measured objectively, although there are putative proxies — emigrant inclinations, for example. But Patten (for it is his responsibility) should be readier than his predecessors to listen to what researchers say. As the whole world knows, people are most easily further demoralized by being told that their assessment of their own states of mind must be imagined.

It also falls to Patten to promise a measure of stability for the British system of research support. Three important changes are now under way [em] the transfer of a chunk of the collective recurrent budget of the universi-

ties to the research councils (which will then accompany research grants with overhead payments), the designation of polytechnics as universities and the balkanization of the system of university support (with separate funding councils for Scottish and Welsh higher education). It remains to be seen what scope there will be for institutional self-improvement under the new overhead arrangements, the redesignation of polytechnics is welcome but precipitate and the balkanization of funding arrangements a needless concession to regional ambitions that oddly conflicts with Major's passionate defence of the integrity of the Union (of the United Kingdom) during the election campaign. Patten could do worse than promise that this will be an end to structural change for the time being.

At some stage, he will also have to find more money, and be content that most of it should be spent on salaries. It is absurd that the chief avenue of recruitment into British research should require PhD students to subsist on less than half the income of a stenographer, but at present there is no choice; students might otherwise be earning more than those who teach them. The research community could help Patten (and itself) by hammering out a tolerable mechanism for deciding researchers' pay. But that will not pay for more research, which is where Heseltine has a role to play. DTI is traditionally responsible for British representation at Brussels, which has more money than good ideas. If Heseltine were to push for a European Community research programme functioning as a grant-making agency, he would win many friends and do a lot of good. □

Healy in a hurry

Dr Bernadette Healy has rid the Human Genome Project of Dr J. D. Watson in a distasteful way.

DR James D. Watson, co-discoverer (with F. H. C. Crick) of the structure of DNA, director of the Cold Spring Harbor Laboratory and of the US Human Genome Project, can be an awkward customer. Unsurprisingly. He has strong opinions. He usually knows what he wants. He is often right. Dr Bernadette Healy, director of the US National Institutes of Health (NIH) for the past year, also has strong opinions and seems to know what she wants. It has been plain for some time that she has wanted



Watson out of the Human Genome Project. Now she seems to have had her way; Watson resigned last week (see page 549). But Healy will find that she has damaged herself more than she has hurt Watson. The Human Genome Project itself will be the chief victim of her impatience.

The way in which Watson has been forced out is discreditable, and is a worry for all who may in future be asked to help out at NIH. That Healy and Watson did not get on has been an open secret for some time. Healy seems to like decisions to be clear-cut, Watson tends to reflective procrastination. For example, he openly disagreed with Healy's support for the NIH plan to seek patent protection for the nucleotide sequences of human genes when nothing was known of their function, chiefly on the grounds that this activity may make a monkey of the Human Genome Project (which it will). That is an issue that NIH should have been willing to talk out with the research community. Instead, Healy has got rid of Watson by fussing about the supposed conflict of interest arising from his ownership of shares in various pharmaceutical and biotechnology companies, potential beneficiaries of the Human Genome Project.

Ends do not justify all means. The means chosen in this case, those of casting a slur on a distinguished helper, even if one chosen before her time, are likely to rebound on Healy. People will wisely think twice before acceding to future requests for help. And what if Congressman John Dingell and his eager committee aides get wind of this whiff of scandal? Neither Healy nor NIH would benefit from the full rigours of congressional control of appointments to the army of advisory committees without which its external functions would collapse. Yet much the same has already happened as a result of Healy's precipitate intervention last year in the affairs of NIH's Office of Scientific Integrity (OSI); she may have had good cause to demand that OSI's procedures should be more formally judicial, but the manner of her removal of Dr Suzanne Hadley has had the effect of transferring control of OSI to the Department of Health and Human Services — and of unjustly delaying several important decisions. Everybody will sympathize with Healy's wish to get things done, but will hope that she learns the benefits of circumspection. Quickly. □

Lost numbers game

The US National Science Foundation (NSF) should apply to its own studies the rigour it expects of grant-applicants.

NSF MADE a sorry mess of its defence last week of a poorly done forecast that the United States will be short of 675,000 scientists two decades hence (see page 553). The chief author of NSF's study, policy analyst Peter House, was obliged to admit to a congressional subcommittee that it was a theoretical exercise without bearing on reality.

To be fair, NSF's fuzzy thinking on manpower is encouraged by the inclination of elders in the research community always to advance bullish estimates of future demand, apparently indifferent to the hundreds of PhDs competing for each academic vacancy and the thousands of lay-offs of skilled people by companies in high technology. Sadly, these arguments stem more from the heart than from the head. The elders are dismayed that the brightest students no longer automatically specialize in science, mathematics and engineering. They fail to recognize their own love of learning in those who choose law or business studies and are saddened that a starting salary of \$80,000 a year on Wall Street should often seem so much more desirable than a post-doctoral fellowship worth, say, \$18,000. They say publicly that the United States needs talented youngsters in science to compete with economic powers such as Japan and Germany, but cannot back up their assertion with evidence.

Of course, there is no accepted yardstick for telling how many scientists a country needs, but even the simple concept of supply is fraught with danger. One reason why the NSF study ran aground was its assumption that the supply of 22-year-olds is a proxy for overall supply when, in truth, there is an untapped pool of millions of scientists in the labour force not at present working in their chosen fields. Moreover, the preferences of 22-year-olds are shaped by crises, real or imagined. For example, the rate of participation in science rose after the Soviet Union put the first satellite (Sputnik) into space in 1957; only a few years earlier, it had fallen precipitously as a result of the glut of talent generated when returning soldiers resumed college education and flowed into technical fields. If women and minorities begin to enter science in numbers closer to their representation in society, supply will rise on its own.

The other half of the labour equation, the demand for scientists, is shrouded in similar uncertainty. The health of the economy, rather than a preference or distaste for scientific talent as such, determines how many technical jobs there are. In the United States just now, less spending on defence has significantly shrunk the technical work-force, for example.

The NSF study disregarded such factors in its search for a single number that might rally support for its cause. Inevitably, it backed into an indefensible position. In doing so, it has given comfort to those who doubt NSF's capacity to carry out analyses of complicated problems.

But there will be lasting and more serious consequences. This fiasco muddies the waters for the next attempt. It also chips away at NSF's credibility when its small but growing budget is under attack from those whose causes, from education to housing, have not been similarly blessed. None of this implies that there are no problems in the recruitment of able technical people, but merely that NSF has missed a chance to find solutions of them. □

Watson resigns, genome project open to change

- Will leave immediately, acting head named
- Smaller centres, more research anticipated

Washington

JAMES Watson, who officially resigned last week as director of the \$3,000 million US human genome project, was probably the only person who could have brought the effort so far in its first three years. But now that the once-controversial project is on its feet, many researchers are hoping for a change of pace.

Advocates of cDNA sequencing, small genome centres and more research on the way genes function — all of whom struggled for funding under Watson's vision of a high-technology genome effort with an emphasis on mapping large stretches of DNA in the human genome and other model species — are likely to see their fortunes brighten under new leadership. And other researchers are hoping that a change at the top may mean an end to the 'old boys' genome network' that they believe has kept the Center for Human Genome Research at the National Institutes of Health (NIH), which Watson directed, from evolving as quickly as similar efforts in other countries and even within the US Department of Energy (DOE), which shares responsibility for the project.

Watson departed in the wake of concern over possible conflict of interest in his holdings of various biotechnology stocks and an increasingly visible dispute with Bernadine Healy, the NIH director. His stepping down is seen as the loss of an able genome advocate and a harbinger of difficult political times. Genome researchers have expressed nearly uniform sadness over the circumstances and haste of his departure. They also credited Watson for giving the project shape in its early years, and leading it through initial congressional opposition.

But it was the timing of Watson's decision that concerns researchers most. He had been expected to leave soon anyway, says Norton Zinder, a Rockefeller University geneticist and former chair of Watson's genome advisory panel. But Zinder and other associates had recommended a graceful departure towards the end of the year, after seeing through this year's congressional budget process. Almost any arrangement, in fact, would have been better than what actually happened: a tumultuous resignation coming just days after the first congressional hearing on the project's proposed 1993 budget.

If the project can survive this year's budget cycle, however, it may emerge reinvigorated. Watson formed a working

enterprise out of what was only an idea four years ago, but "he never planned to stick around to micromanage the genome project," says Zinder. But, over the past year, some researchers were concerned that that was just what was happening. Watson was more of a genetic visionary than a practising researcher or manager, and his strength, both at the genome project and as director of the Cold Spring Harbor Laboratory on Long Island, New York, was not in day-to-day operations.



Watson's departure may make a place in the genome project for cDNA and its chief advocate, Craig Venter (at right, with NIH's Mark Adams)

His clash with Healy over cDNA patents last year was only the most apparent of his legendary disagreements with members of the genome community. He also opposed cDNA sequencing itself, arguing against churning out portions of expressed genes without knowing their function. And his determination to focus on obtaining a physical map of the entire genome assumed that gene sequencing technology would see great improvement over the past few years, something that has not happened.

"According to his text, we'd get revolutionary improvements in the technology, and that has not materialized," says Paul Berg, director of the Stanford University Beckman Center and current chair of the genome project's advisory committee. Sequencing cDNAs offered a cheap and easy way to find expressed genes that could be used as markers in genetic mapping, and an alternative to the straight-ahead, sequence-to-the-end approach that Watson advocated as the eventual goal.

But Watson disapproved of cDNA sequencing as being insufficiently rigorous,

and he fought its leading proponent, J. Craig Venter of the NIH. Since then, the DOE (which supports about a third of the US genome project), France, the United Kingdom and Japan have all embraced cDNA sequencing. Only NIH have resisted.

Now, says Berg, "I think that there will be a much more receptive atmosphere to cDNA work." In his effort to give the genome project direction and momentum in the face of early opposition, Watson "may have focused too narrowly", Berg says. "He wanted to keep people's noses to the grindstone. And we might not have been so far along today, if it were not for his single-mindedness and doggedness." Berg predicts that the genome project will in future be more tolerant of other approaches, including more studies of gene function and biology.

Small genome centres may also come into favour. Several teams in Europe that are studying the yeast chromosome have shown that a dozen researchers with an automated gene sequencer, if they collaborate will similar teams, can be as productive as a large group, says Venter. "Originally, it seemed like exactly the wrong approach — exactly the opposite of the high-tech strategy" that Watson advocated, he says. But once the small teams learned to work with each other, they were able to move on to different projects with a flexibility that large laboratories can only hope for.

NIH are deciding how large to make the next set of genome centres. Many researchers argue for a balance between large and small laboratories, rather than a focus on laboratories with a 'critical mass' of researchers, about 20 PhDs, such as is headed by Eric Lander at the Whitehead Institute of Biomedical Research in Cambridge, Massachusetts.

In his resignation letter, Watson promised to continue to support the project enthusiastically, and to advise NIH informally. But his resignation takes effect immediately, and last week Healy appointed Michael Gottesman, currently chief of the Laboratory of Cell Biology at the National Cancer Institute, to be acting head of the National Center for Human Genome Research.

Healy also announced that the search for a permanent director would begin immediately. Although several prominent researchers (including Victor McKusick, a geneticist at Johns Hopkins University) have been mentioned in the past as possible replacements for Watson, the likeliest candidate right now is Daniel Nathans. He is a Nobel Laureate like Watson and, like McKusick, a Johns Hopkins geneticist, as well as being a member of the President's Council of Advisors on Science and Technology, on which Healy served before becoming director of NIH.

Christopher Anderson

Japanese debate more or better links

Tokyo

JAPANESE researchers who would like to expand a computer network to many more universities are lining up against a powerful government agency that wants instead to improve service to a smaller number of existing sites.

The scientists would like to see more regional networks that would connect smaller universities and research institutes in the more remote regions of the country with high-speed access to international scientific networks. But the powerful National Centre for Science Information Systems (NACSIS), which oversees the domestic development of computer networks in Japan's universities, wants the government to concentrate instead on a super-high-capacity computer link between the Kanto region around Tokyo and the Kansai region around Kyoto and Osaka, where most of Japan's research activity is concentrated.

Last week, Tsuneyoshi Kamae of the physics department of Tokyo University invited a handful of leading information scientists from Japan's universities and industry to an informal meeting to discuss how to develop such a regional computer networks. In 1989, Kamae almost single-handedly established the Todai International Science Network (TISN), a high-capacity computer link with the West that was Japan's first such link for general scientific use (*Nature* 340, 670; 1989).

TISN has expanded rapidly despite having no official backing of any government ministry or agency. Users simply pay \$10,000 towards the international link to Hawaii and install their own domestic link to TISN in Japan. Beginning at Tokyo University, TISN now connects nearly 20 of Japan's national universities and research institutes in a network that provides a 64-kilobit-a-second link to the University of Hawaii. From Hawaii, researchers gain access to scientific networks around the world.

Among the most recent groups to join TISN are Japan's genome researchers. Researchers belonging to the human genome project of the Ministry of Education, Science and Culture (MESC) have linked TISN to their genome network, which connects computers in Osaka University, Kyoto University and the National Institute of Basic Biology in Okazaki to the new human genome research centre

in the Institute of Medical Science in Tokyo University. And they plan to extend the genome network to Kyushu University in Japan's southern island of Kyushu.

Rice genome researchers working under the Ministry of Agriculture, Forestry and Fisheries also plan to tap into TISN. And human genome researchers under the

MESC in Tokyo in 1986 to help build computer links among universities. With an annual budget of about ¥3,000 million (US\$22 million), NACSIS has placed hundreds of computers and packet switching nodes in universities all over Japan and linked them with high-speed digital circuits.

But NACSIS is handicapped by its decision to use a little-known protocol called N-1. N-1 was developed domestically in the 1970s with a grant from MESC. But computer networks that use the N-1 protocol are unable to communicate with computer networks outside Japan that use other protocols, in particular the widely used TCP/IP protocol.

In January 1989, NACSIS opened with much fanfare a high-capacity computer link with the National Science Foundation (NSF) in Washington. At the time, Japanese officials said it would open up the country's scientific information resources to the West. But the NSF link is essentially a dead end: because of protocol and other incompatibilities, US computer users cannot access it.

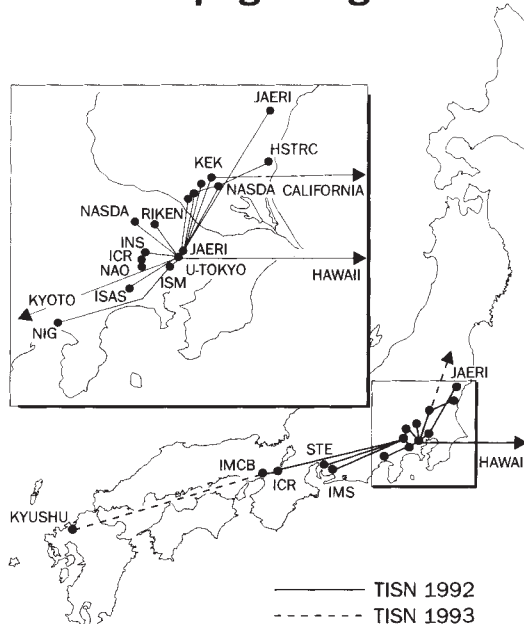
Shoichiro Asano of NACSIS admits that the NSF link has not been put to much use. He says that his centre has just established another high-capacity link to the United States that uses the TCP/IP protocol and that the NSF link will be "removed". NACSIS has also been gradually converting its domestic scientific network to the TCP/IP protocol over the past few years. And NACSIS will this month open a backbone TCP/IP

network of nine new nodes, called SINET, that links Sapporo in the northern island of Hokkaido to Hakata in Kyushu.

While this switch to TCP/IP is welcomed by Japan's scientists, there is disagreement about what should happen next. Kamae and other pioneers of networks in Japan, like Shoichi Noguchi, director of the Research Centre for Applied Information Sciences in Tohoku University, would like the government to invest in regional computer networks so that small local universities and research institutes can tap into the new high-speed networks.

"I can communicate much more quickly and efficiently by computer with Oxford University in the United Kingdom on the other side of the globe than I can with Gumma University just outside Tokyo", says Kamae. Many small regional univer-

Japan's international computer network keeps growing



JAERI Japan Atomic Energy Research Institute, **KEK** National Laboratory for High Energy Physics, **NASDA** National Space Development Agency, **NAO** National Astronomical Observatory, **ISAS** The Institute of Space and Astronautical Science, **NIG** National Institute of Genetics, **IMS** Institute for Molecular Science, **STE** Solar-Terrestrial Environment Laboratory, Nagoya University, **ICR** Institute for Chemical Research, Kyoto University, **IMCB** Institute for Molecular and Cellular Biology, Osaka University, **RIKEN** Institute of Physical and Chemical Research, **INS** Institute for Nuclear Study, **ISM** The Institute for Statistical Mathematics, **HSTRC** Hiraiso Solar Terrestrial Research Centre.

Science and Technology Agency, who are planning to link the Japan Information Centre of Science and Technology (JICST) to the Genome Data Bank (GDB) in Johns Hopkins University in the United States, also intend to link JICST to TISN so that all of Japan's genome researchers can access GDB.

Paralleling the development of TISN is another high-capacity computer network called the Widely Integrated Distributed Environment (WIDE). Established by Jun Murai of Keio University with donations from Japanese industry, WIDE is intended primarily for those who are developing computer networks. But university researchers also use it to gain international access to overseas computer networks.

Lumbering along behind these pioneering efforts is NACSIS, established by

sities like Gumma still only have 9.6-kilobit links to the NACSIS scientific inter-university network. In addition, the regional universities lack local area networks on campus, and the national universities are acquiring them only slowly, at a rate of one or two a year.

Asano of NACSIS admits that the issue of regional networks is "very political". But he argues that it is premature to establish such networks because there is a lack of "human resources" to manage them. A central management system is needed, he says, a job that the centre is starting to take on. In the meantime, NACSIS is concentrating on a plan to establish a six-megabit, super-high-capacity link between Tokyo and Kyoto.

But Kamae and his colleagues see no need for it. "We can easily supply that route with existing networks", Kamae says. "What is needed now is a bottoms-up approach".

It is not clear how such disagreements will be resolved. But even if NACSIS goes ahead with its plans, as seems likely, Kamae and his colleagues have already shown a remarkable capacity to establish links outside the NACSIS system.

David Swinbanks

Who pulled the plug?

UNIVERSITY scientists outside Japan who try in December to communicate via computer with their Japanese counterparts should not be surprised if the line suddenly goes dead for a few days. The silence is a result of government auditors trying to save the taxpayer a few yen.

Every year the National Centre for Science Information Systems (NACSIS) pulls the plug on the nation's university computer network to allow telecommunications carriers to bid on a contract to operate the system. Japan has several domestic telecommunications companies whose rates vary. And government financial auditors insist that the carriers must bid every year to provide the cheapest telecommunications links for NACSIS's nationwide computer network. To allow fair bidding, no carrier can provide telecommunication links for the computer network during the bidding process, which takes several days.

Last December, Japan was cut off from the outside scientific world for as much as a week. Shoichiro Asano of NACSIS says he is trying to shorten the bidding process. The centre also plans to install a back-up system using ordinary telephone lines so that the network remains open even during the bidding.

D.S.

NSF pays price for success

Washington

WIDESPREAD acclaim, heady funding increases and thousands of satisfied scientists would be a dream come true for most science projects. But for the five-year-old computer network initiative of the National Science Foundation (NSF), success has also meant a lot of headaches.

The initiative, called NSFNet, has grown steadily since it was created in 1987 as a nationwide 'backbone' research network. Earlier this year, the Administration requested more than \$45 million in 1993 for the agency's networking efforts, an increase of 38 per cent. In December, President George Bush signed legislation creating a National Research and Education Network (NREN) based on the NSFNet backbone. With annual funding expected to reach \$1,000 million by 1996, NREN intends to connect not only research institutions but also schools and industry to a electronic web operating at 1,000 million bits per second.

It did not escape the notice of big business that all that money and responsibility was going to a little science agency. Long-distance telephone companies such as US Sprint also want to get into nationwide computer networking, and they complained bitterly that NSF had essentially handed over a potentially lucrative market to a handful of companies. The main contract had gone to Merit, Inc. (a non-profit consortium of Michigan universities) and Merit's subcontractors MCI and IBM, which handle the fibre optic lines and the routing hardware respectively. The three contractors then formed a fourth entity, Advanced Network and Services, Inc. (ANS), to handle network operations.

In its original plan, NSF had planned to 'recompete' its five-year contract with Merit in November. Normally, that would mean a simple request for applications and a selection of the winner. But pressure on the agency from the telecommunications industry has forced it to abandon its usual methods. Last year, it decided to extend the current contract by 18 months so that it could come up with a plan that would be fair to all concerned.

In the next week or two, NSF plans to issue a proposed solicitation for public comment. After weighing the comments, it will issue a final solicitation, and make awards in April 1993. Rather than retaining a single main contractor, NSF will make at least two awards for the lines and a third award for the routing equipment.

The process is a far cry from the type of small grants that NSF usually awards. "It keeps me up at night", admits Steven Wolff, director of the agency's network division.

Although congressional staff credit Wolff with making the best of a situation that simply got out of control, others say

that NSF will not for long be able to balance the research community with the telecommunications industry. Some predict that NSF will be forced to privatize the network within a few years. "This thing has been growing even faster than NSF expected — they just ran up against telecommunications policy," says Frederick Weingarten, executive director of the Computing Research Association, a coalition of research universities.

Weingarten traces the problem to the mandate in the NREN legislation to serve society, not just the research community. "Once the [Administration] got the word 'education' into the title, it became tied up with a much bigger vision of building a national [network] infrastructure," he says.

When the telecommunications companies saw the scope of the legislation and the role that NSF would play in the network's development, they asked why a science agency — not to mention the US government — was in essence building the next telephone system. At a hearing in March before the House Science, Space and Technology subcommittee on science, industry officials complained that the NSF contract to Merit, IBM, MCI and ANS would allow those companies to essentially monopolize what promised to be a nationwide telecommunications network far beyond the research community.

"The entire group, which is now publicly self-characterized as a 'partnership', has total control over the \$50 million NSFNet backbone contract ... and [is] well positioned to win any NREN contracts in the future," complained William Schrader, president of Performance Systems International, a network services company.

Wolff acknowledges that the plunge into telecommunications policy has been a shock to NSF, but he is optimistic that the recompensation will allow the agency to continue to serve researchers and give companies a chance to compete. The government entered the field, he says, because scientists were not perceived to be a sufficiently lucrative market.

Once the recompensation is completed, NSF's next problem will be to know when to bow out. The network will someday connect commercial databases, schools and companies, and, if the lack of agreement among industry forces the agency to run the network for another five years or so, NSF may find it difficult to keep the focus on the research community.

On the other hand, giving it up too soon may leave researchers adrift. "NSF's not the right agency to run a nationwide network," says Weingarten. "But when it's finally taken out of their hands, I hope [the new owners] don't lose the ability to serve the research community as well."

Christopher Anderson

Academy approves, critics still cry foul

Washington

THE current procedure for comparing DNA fingerprints is "fundamentally sound" and should be considered reliable when done properly, according to a report released this week by the National Academy of Sciences' National Research Council (NRC). But while the report should make the controversial technique more acceptable in the courtroom, it is not expected to end debate about its use.

The report* suggests additional studies and measures to improve the standards of laboratories that carry out the tests and to strengthen the statistical basis for making comparisons of DNA samples. The NRC panel recommended that researchers take DNA samples from 100 people in each of 15 to 20 ethnic groups and that they maintain a database of the blood samples to reduce the possibility that ethnic subgroups in populations can distort the chances of finding random matches. It also recommended that the US Department of Health and Human Services establish an accreditation programme to check the quality of forensic laboratories.

None of these suggestions are contentious. But its chapter on population genetics seems likely to renew debate over whether the technique is a good way to identify someone conclusively.

The US Federal Bureau of Investigation (FBI) and many prosecutors have used a 'multiplication rule' to determine the odds of random matches. Using that technique, a laboratory comparing DNA samples at several locations on the chromosome would multiply together the known frequency of each DNA site in available databases. Critics, however, argue that this technique discounts the possibility that the patterns may be related and inherited together. But in the absence of extensive databases on ethnic subpopulations, researchers do not know which patterns are typically inherited together.

The NRC report compares the multiplication rule to multiplying the odds of finding someone with blond hair by the odds of finding someone with blue eyes to determine the odds of finding someone with blond hair and blue eyes. That approach is misleading, of course, because blond hair and blue eyes are related; the actual odds of finding them together are much higher. The report cites one occasion in which the multiplication method was compared with an actual counting of matches in the database: the first predicted the odds of a match at 1 in 739,000 million, while the second showed the real odds to be 1 in 500.

The NRC report calls for more samples and better databases to reduce reliance on statistical crutches such as the multiplication rule. But it cautiously endorses the

use of a modified multiplication method, using the lowest observed odds for each DNA site.

This compromise, say critics, is just what the DNA fingerprinting advocates wanted. While agreeing with the NRC recommendations on additional measures, the critics claim that the NRC panel acceded to pressure from the US Department of Justice and the FBI, two sponsors of the report. They allege that FBI scientists, to whom a draft copy of the report was leaked last year, convinced the panel that it should delete portions of the population genetics chapter that would have made DNA fingerprinting evidence more difficult to use in court.

Earlier this month, a judge in Seattle, Washington, ordered a copy of the earlier draft of the report released as part of a case (*State v. Copeland*) in which DNA fingerprinting evidence was being challenged. There are substantial changes between the draft (dated 15 October 1991) and the final report, mostly in favour of the use of DNA fingerprinting in court.

In particular, the draft does not recommend the use of the multiplication rule. Instead, it warns that "neglecting population substructure plays the same role in genetics textbooks as neglecting friction and air resistance in physics textbooks."

But by eventually accepting the use of a modified multiplication method (even reluctantly and temporarily), that is exactly what the NRC panel has done, says Peter Neufeld, a lawyer who has fought the forensic use of DNA fingerprinting in several court cases.

Victor McKusick, a geneticist at Johns Hopkins University who chaired the NRC panel, acknowledges that John Hicks, an FBI scientist, submitted a lengthy criticism of the population genetics chapter to the panel. But the main changes in the final version "came out of the review process, not Hicks' letter," he says. As a sponsor, the FBI is not supposed to dictate the final product. But it is not inappropriate for FBI scientists to offer expert advice, he says. (In fact, the preface to the report thanks Hicks for his assistance.) "It is, however, unfortunate that the draft got such wide distribution," McKusick adds.

The controversy over the population genetics is unlikely to die with the release of the report. But McKusick hopes that the recommended research on ethnic subpopulations will ease some of the concerns about the multiplication method, if not the suspicion of political meddling in NRC reports. **Christopher Anderson**

* DNA Technology in Forensic Science, National Research Council, 1992.

OZONE DEPLETION

Mixed Arctic results

London

EUROPEAN scientists measuring the amount of ozone in the stratosphere above the Arctic face a situation in which less is more. While their data show a decline in the thickness of the ozone layer from last year to this, the drop is less than had been predicted last autumn by US scientists using different methods. The result is a mixed message to the public, and a reminder that scientists still have much to learn about the factors that influence stratospheric ozone depletion.

The European Arctic Stratospheric Ozone Experiment (EASOE), which ran from November until March, was designed to swell scant knowledge of the atmospheric dynamics of the north polar regions. The preliminary results showed a complex situation, with ozone loss from halons aggravated by the aerosols from the eruption last summer of Mount Pinatubo and the huge high-pressure system that has lurked over Europe for much of the winter. The effect worsens over the winter and dissipates once spring arrives.

A team of scientists have so far found decreases from historical levels of between 10 and 20 per cent in stratospheric ozone. Although that is greater than ex-

pected, it falls short of predictions by a team of scientists at the US National Aeronautics and Space Administration of declines approaching 40 per cent. That estimate was a 'worst case', with all the active chlorine measured in the atmosphere over the Northern Hemisphere being involved in ozone depletion.

Less work has been done on Arctic ozone than on the situation over the Antarctic, where a hole was discovered in the mid-1980s. And the differing meteorology at the two poles makes comparisons difficult. A tight polar vortex in the Antarctic produces sustained low temperatures and inhibits the exchange of air, whereas the Arctic has a looser and warmer vortex. The EASOE team hopes to learn much more about polar dynamics and the behaviour of active chlorine in the next few months as it analyses the data.

The experiment covered a wide area stretching from central Europe, across the North Atlantic to Greenland and the Russian Arctic, and involved more researchers, working for a longer time on more experiments, than ever before. "The collaborative nature of the campaign was very successful", says the project's coordinator, Neil Harris. **Ian Mundell**

NSF falls short on shortage

Washington

A WIDELY publicized study by the National Science Foundation (NSF) forecasting a shortage of 675,000 scientists in the next two decades is so flawed as to be nearly worthless, a subcommittee of the US Congress has concluded. While the committee's year-long investigation into the issue — which culminated in a six-hour hearing last week — may have sullied NSF's reputation, it leaves unanswered the larger question of whether the nation is training a sufficient number of scientists to meet its needs in the next century (see page 548).

At issue is a 1987 study by NSF policy analyst Peter House. It reaches the simple conclusion that a declining number of college-aged students also will mean fewer graduates trained in science and engineering. House drew an imaginary line from the record high level of science degrees awarded in 1984-86 and calculated the difference between that hypothetical level and the actual number of degrees awarded.

But labour economists and policy analysts say that the resulting number — 675,000 between 1986 and 2006 — is meaningless. It cannot be called a shortage, they say, because a shortage requires an estimate of demand, which House did not attempt. In addition, the assumption that those graduates represent the supply of future scientists also ignores such factors as the existing labour pool of scientific talent working outside their field, the migration of workers into science and any changes in the proportion of undergraduates who choose to major in science. And because the number is an aggregate, it could mask important trends within specific fields. In short, say those experts, the number is wholly useless for its original purpose as a policy tool.

"The long-term accuracy of such predictions isn't sufficient to guide federal policy," testified John Andelin, an assistant director at the congressional Office of Technology Assessment. Asked about the wisdom of offering a single number, Andelin said, "if you want press attention, yes, it's useful. If you want intelligent discourse, the answer is no."

But that did not stop NSF from using it, according to the chairman of the investi-

gations subcommittee of the House science committee that called the hearing. Representative Howard Wolpe (Democrat, Michigan) says that NSF officials, including its former director, Erich Bloch, cited that number repeatedly in testimony and speeches warning of a looming, serious shortage of scientists. The number helped NSF to gain a larger budget, Wolpe claims, and became part of legislation. For years, NSF officials stood by the

number despite strong criticism from inside the statistical community, Wolpe pointed out.

Although such tactics are commonplace in Congress, he added, "nobody expects NSF to play that game. Everyone around here assumes that NSF's numbers are good science."

House defended his study as nothing more than a theoretical exercise to understand the impact on science

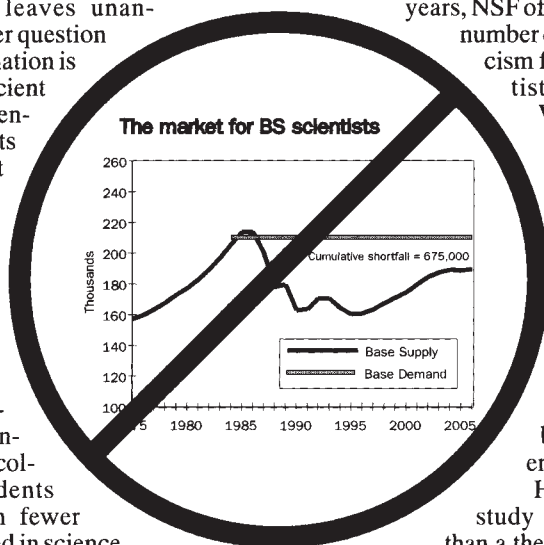
of the so-called 'baby bust' of the 1970s that followed the baby boom after the Second World War. He said he never intended it to be used as policy document and had "no way of knowing" whether it influenced policy-makers either inside or beyond NSF. The projected difference between the number of degrees awarded in the mid-1980s and the next two decades should be called a 'shortfall', he said, not a shortage.

Wolpe said that he found House's explanation "extraordinary". Referring to previous panels of experts that had attacked House's study as "pseudoscience" and "nonsense", Wolpe asked House at one point, "So you're saying that everybody who has testified here today wasn't understanding your study?"

Wolpe was also troubled by NSF's reluctance to acknowledge the persistent criticism of the House study. He said that internal NSF memoranda that refer to "protect[ing] the foundation from damage" and "losing this discussion" suggest that NSF officials were defending their mistakes rather than trying to correct them.

Walter Massey, who succeeded Bloch as director of NSF in March 1991, assured Wolpe that that was not the case. "I treat this as a very serious matter", Massey said, pointing to a new round of grants for research to improve the quality of the data on factors that affect scientific supply and demand.

Jeffrey Mervis



A European university

London

EUROPE needs a unified university system to solve the scientific and technical problems of the next century. The idea, offered at a conference last weekend on "Europe in the Third Millennium", came from Kai Simons of the European Molecular Biology Laboratory in Heidelberg and was received warmly by academics, civil servants and politicians. The event was organized by the Centre for European Studies at the University of Exeter.

"The present state of science in European universities is not encouraging," Simons says. "Piecemeal delivery of teaching without coordination, proliferation of sub-areas leading to narrow-minded specialization and lack of mobility, accompanied by stagnation and local in-breeding, are conspicuous ingredients in the activities of many science faculties. This lack of communication results in myopic research, filling scientific journals with papers that no one reads."

Simons suggested that a number of model faculties should be created to teach an integrated curriculum to students from around the world. Upon graduation, they would carry some sort of European quality stamp. "The intellectual effort to do this should come from the universities themselves, but the stimulus should come from Brussels," Simons said.

Although the participants supported the notion, there was some doubt about how effective such involvement might be.

"We need a committee in Brussels to pick the institutes which would become the models," commented Sir Eric Ash, rector of Imperial College, London, "but doing this would limit the freedom of the institutes to achieve the changes."

Others pointed to the success of informal agreements that have arisen below the bureaucratic water mark. At present, the European Commission cannot intervene in education policy as it can with research. However, programmes such as Erasmus and Tempus — designed to facilitate an interchange of students within Europe — have had considerable influence on European education.

"Erasmus is a multiplying force for many things, such as curriculum change, which were not its purpose," said Theo Veenkamp, director of the European Community's Tempus office. "If it had been set out explicitly, there would have been a political uproar. We need innocent impulses from Brussels that will also act as a catalyst for the sort of changes we want, and let them evolve naturally," he said.

Such a scheme would cost a great deal, the conferees agreed. But they had plenty of suggestions about how to pay for it, including taking money from the EC's Common Agricultural Policy or the US manned space programme. Ian Mundell

Congress attacks contractor, accountants

Washington

ALTHOUGH supporters of the Superconducting Supercollider (SSC) insist that the massive particle accelerator is being built "on time and under budget", a congressional investigation revealed last week that the Texas project still has more than its share of financial and management problems.

Investigators have documented a power struggle between the managers of the SSC laboratory and the private building contractors that could put the project millions of dollars over its estimated cost of \$8,255 million and months behind its 1999 completion date. But the impact of the clash remains an open question because the Department of Energy (DOE), which oversees the project, has admitted that it has no effective accounting structure.

At a hearing last week before the oversight and investigations subcommittee of the House Science, Space and Technology Committee, the congressional General Accounting Office (GAO) testified that the SSC is reaching its challenging technical milestones without much trouble. But the same cannot be said of its 54-mile elliptical tunnel and accompanying scientific buildings.

Early in the construction process, officials were forced to move the Magnet Development Laboratory after geologists discovered an aquifer directly under the original site. Racing to regain lost time, builders started construction before its design had been finished. When officials realized that the laboratory would be too small, they had to rebuild it partially and expand it.

A second snag arose from a new analysis of the underground site for the large experimental halls that will contain the SSC's largest detectors. Project managers decided that the rock on the other side of the ring was better suited for tunnelling. GAO testified that this could delay the project by 13 months and add \$400 million to the cost.

DOE officials justify the switch by saying that it would have taken even longer for geologists to decide how to keep the experimental halls on the west side of the ring, as originally planned. And although the start of construction will be delayed by the change, DOE thinks that it can get back on schedule by such approaches as designing the experimental halls while they are being built (despite the failure of that approach with the magnet laboratory) and building only one large experiment hall rather than the two now planned.

Strained relations between the laboratory and its construction contractors, Parsons Brinckerhoff-Morrison Knudsen (PB/MK), have further complicated matters. Some cost estimates have turned out to be too low after the contractor cut corners without authorization. In one case,

PB/MK decided unilaterally to extend by 10 metres the spacing between some 150 equipment 'niches' in the tunnel. The change would have allowed it to build three fewer niches.

Other cost estimates have been found by the GAO to be too high after both parties included the same portion of the project in their own plans and budgets. Russ Wylie, the SSC's director of external affairs, blames the problem on an "ongoing difference of opinion between PB/MK and the lab over the scope of the services". Several attempts to restrain the contractor over the past year were unsuccessful, but Wylie insists that the situation has changed.

A cost estimate on 1 September by SSC officials put the most optimistic forecast at \$73 million over the \$1,500 million "baseline" construction plan. A "pessimistic" forecast has the cost exceeding the baseline by as much as \$383 million.

Although the officials did not report these figures to DOE, some of the other problems were already becoming evident. Henson Moore, then DOE deputy secretary, warned in a letter dated 24 January to Roy Schwitters, the SSC director, that construction management appeared to have got out of hand and might call for legal action. "I have learned that the overrun problems are continuing and may even be getting worse," Moore wrote. He said that he was "extremely upset at this news" and demanded a plan to bring SSC construction back under control.

On 1 April, after negotiations with the contractor, SSC officials recalculated their

costs. Instead of assured overruns, the new estimate gives a range of \$300 million, from a saving of \$142 million to an overrun of \$160 million. Critics quickly dubbed it the "April Fools' Day forecast" (noting that a yearly "escalation factor" had been arbitrarily set at 3.5 per cent less than previous estimates), but DOE officials explained that most of the savings came from removing parts of the project that the contractor had wrongly claimed as its own responsibility.

Although neither GAO nor the subcommittee's investigators has been able to refute DOE's estimates, each is convinced that the forecasts are unrealistic. Part of the problem, GAO testified, is that DOE's accounting methods make it nearly impossible to predict the impact of design changes and delays. DOE says that it has adopted better accounting and management procedures.

But getting to the bottom of the SSC's accounts has been complicated by what subcommittee members describe as a concerted campaign by DOE to avoid outside scrutiny. "Documents have been withheld, information shared only sparingly and there have even been attempts to pull strings to stop our oversight activities," said Representative Sherwood Boehlert (Republican, New York). "Never have so many done so much to avoid the scrutiny of so few."

Last year, the subcommittee threatened to issue a subpoena when it could not get the documents it wanted (see *Nature* 354, 259; 1991). The panel hopes that DOE will be more cooperative after being scolded in public.

Christopher Anderson

ANIMAL RESEARCH

Military stands firm on dissection

Washington

ALTHOUGH many US universities now offer alternatives to animal dissection in medical training, military officials stuck by their guns in a congressional hearing last week and refused to offer exemptions to students who seek such alternatives.

Officers from the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, Maryland, explained that their students agree upon admission to dissect animals as part of their training. University officials said that they do not believe that animal alternatives, such as computer simulations, are adequate substitutes for the use of animals by students.

Last year, Representative Ron Dellums (Democrat, California), chairman of the research and development subcommittee of the House Armed Services Committee which held last week's hearing, and ten other Members of Congress wrote USUHS asking the university to explore non-animal

alternatives and to allow students to be exempted on religious or ethical grounds. USUHS officials said no.

Elsewhere, the dissection issue has generally been handled on a case-by-case basis at US universities. The American Medical Association supports offering alternatives to students who choose not to participate in animal dissection. Several US universities, including the medical school at the University of Maryland, have eliminated animal laboratories from their curricula.

Military officials at the hearing also defended their use of animals in research against criticism from six animal-rights and animal-welfare organizations that offered examples of what they considered to be abusive and wasteful research. But Dellums is not convinced. He said that he might call another hearing, closed to the public, to review classified military research on animals.

Christopher Anderson

Healy attacks US budgeting system

Washington

THE director of the US National Institutes of Health (NIH) thinks that the federal government, including her political bosses in the White House, should find a better way of deciding how to spend its money on science. Her comments add to the growing debate about allocating scarce resources among increasingly expensive research projects.

Testifying last week before the Committee on Science, Space and Technology of the US House of Representatives, Bernadine Healy complained that "I don't think that we have a mechanism within the executive branch that looks at science priorities". She said that the heads of the dozen or so agencies that pay for most federally funded research never have the chance "to sit around a table and let it all hang out", and that the relative merits of major scientific projects in different fields are never examined.

"Should science have the equivalent of military base closings?" she asked rhetorically. The current system is not designed to ask that sort of question, she replied.

Healy was especially critical of the government's chief tool for that purpose, the Federal Coordinating Council on Science and Technology (FCCSET). "FCCSET is not a policy-setting group. Its members don't have enough clout." And she said that NIH are "left out of most of

the high-level debate on science policy" despite the fact that her agency funds half of all the academic research supported by the federal government.

Last week's hearing was the first in a series that the subcommittee plans to hold this spring on setting priorities for the federal funding of science. The end of the Cold War has generated pressure to shift the government's \$70,000 million investment in science and technology, now weighted toward defence, to the civilian side. The scientific community is also debating the impact of such costly projects as the space station and the Superconducting Supercollider (SSC) on maintaining an adequate level of support for individual investigators. Those tensions have already spawned reports by foundations, professional societies and government agencies on ways to improve the current budgeting process.

Sitting next to Healy at the witness table was Walter Massey, director of the National Science Foundation (NSF). It was the first time that the heads of the government's two leading agencies for funding basic science has appeared together before the science subcommittee and the first time Healy had been asked to testify before it. But their proximity did not lead them to speak with one voice.

Massey toed the administration's line, saying that FCCSET does a good job of

bringing together policy-makers from the various research agencies. "You don't need to create another mechanism", he told Representative Rick Boucher (Democrat, Virginia), chairman of the subcommittee. He suggested that Healy's frustration with the process stems in part from the fact that her agency is represented within FCCSET by its parent, the Department of Health and Human Services (HHS).

But Healy was not finished. She criticized the White House's Office of Science and Technology Policy (OSTP), which oversees the various FCCSET panels, for traditionally "ignoring" NIH and the life sciences. Part of the problem, she said, is that NIH's mission to improve the public health through research means that it "isn't seen as a science agency".

Massey reminded the subcommittee that it was a waste of time to talk about priorities in science without including the Office of Management and Budget, which reviews (and usually reduces) the budget requests of every federal agency. That process pits NIH against the rest of the HHS budget, including such popular social service programmes as Head Start, a pre-school programme for disadvantaged children. "We are not compared with NASA or NSF or the SSC" Healy explained. Asked later if she would prefer to compete against those projects, she replied, "Sure, if it was a fair test".

Jeffrey Mervis

INDUSTRIAL RESEARCH

Report calls UK approach simplistic

London

THE reliance of the UK government on a pipe-line approach to funding civil research is based on a discredited model of innovation, according to a report from the University of Cambridge.

During the 1980s, the government progressively withdrew from funding what it saw as near-market research in industry, on the theory that market forces could best dictate research priorities. Instead, the government concentrated its resources on pre-competitive research, altering the mechanisms by which policy was decided so that it had more control over what work was done.

The result, says the report, is a government policy that is out of touch with reality in both the research laboratory and the shop floor. "It is ironic," says Elizabeth Garnsey, one of the authors of the report, "that the government sees basic science as more predictable than innovation carried out by industry."

According to the report, the government misunderstands how industry react to incentives to invest in research. Although normally only large, established

companies have the resources to engage in long-term research, they do not necessarily benefit from the disruption to established markets that innovation invariably causes. To protect their interests, market leaders stifle the flow of ideas by engaging in such practices as pre-emptive patenting.

By the same token, the small companies that were fittest to innovate and stand to gain the most from disrupted markets do not have the funds for long-term research. Not only has the government's approach deprived them of direct funding for near-market research, but administrative complexity has excluded them from more recent collaborative programmes. In addition, many publicly funded research institutes have been privatized, reducing the amount of research in the public domain.

The pipe-line approach to innovation assumes that ideas from basic research flow into industry, which then adapts them for the market. Conventional wisdom among science policy researchers prefers a spiral to a linear model: "All the empirical evidence indicates that innovation is an interactive and iterative process, and one which is frequently user-led. The interactions oc-

cur between those initiating technological ideas, those with opportunities for new technological applications and those with user needs", the report says.

Recent adjustments to the LINK programme for industrial/academic collaboration in the United Kingdom, and the launch of the £32 million (US\$55 million) SPUR scheme (see *Nature* 349, 556; 1991) to fund near-market research, indicate that the Government recognizes some of the inadequacies of its approach. However, these changes fall short of what the report feels is necessary.

In a separate document, the group recommends that the government's highly successful SMART award scheme, which funds near-market research in small companies, should be expanded so that it makes awards twice a year, rather than just annually, and rewards all worthy applicants.

Starting with £29 million in 1989 to cover the four-year scheme, the programme has received more money each year as the number of applicants increases. Expanding the scheme as the Cambridge report suggests would mean a dramatic increase in funding.

Ian Mundell

Measuring animal well-being

SIR — In his Commentary "Today's non-Orwellian animal farm"¹, Jukes claims that the productivity of farm animals is an index of their well-being and that, because economic success for intensive agriculture is measured by productivity, the well-being of the animals involved is protected.

It is true that a sudden fall in productivity may indicate a welfare problem, but welfare cannot simply be measured by productivity. The definition and measurement of animal welfare have been studied and described extensively and several comprehensive reviews are available²⁻⁴.

One problem with using productivity to measure welfare is that the welfare of an animal is a property of an individual, but productivity, particularly in intensive agriculture, is usually measured in terms of flock or herd production. Another problem is that one measurement may

give different results for animals of different individual value. For example, a mortality rate of 7 per cent is not unusual in rearing veal calves to six months of age and appears to be acceptable to many farmers. The same mortality rate would not be economically acceptable to a farmer rearing valuable dairy replacement calves.

The major problem, however, is to decide which measurement of productivity to use. To quote from a review of this problem in respect of poultry⁵, "a change in an environment variable may reduce the number of eggs produced, but increase egg weight, leaving egg mass output the same. Depending on the measure of productivity selected, the change could be said to be an extreme example, but in the course of investigating the welfare of several species of farm animal over the past few years, I have found that producers do indeed use such arguments.

For example, laying hens kept in battery cages often have poor feather cover and poor skin condition but high rates of egg production. Foxes kept in cages usually have good fur and skin condition but often have low reproductive rates. I have been earnestly informed by battery cage producers that egg production is the only real measure of a hen's welfare and that skin and feather condition are irrelevant, whereas fox farmers tell me that reproductive rates have little or nothing to do with welfare but a good shiny coat is a true indication of a happy fox (presumably as long as the fox is still wearing it).

I am more than a little surprised to find a professor of biology giving credence to this folklore, even if, as he says, he worked on a farm in the 1920s.

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SIR — Jukes questions the notion that animals feel better when they have more space for roaming (his argument: "how do we know this is true?"). He should ask himself why chicken get their beaks cut and pigs their canine tooth removed — not because the animals smile too much but because they attack each other under their crowded stress situation. Why did Jukes compare the situation of these animals jammed in cages with crowded human gatherings such as watching sports where the participants

come for a short time and leave as they please? Why not, with respect to the impossibility of escaping and imminent death, compare it to Hitler's concentration camps? Jukes argues further that heavy drug use in farm animals is necessary because the prevention of disease also prevents suffering. He should have mentioned that those animals are much more prone to diseases because of the conditions in which they are raised.

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SIR — I agree with Jukes that it is unfair to allege that people engaged in farming are oblivious to animal welfare, but it is certainly not unfair to question the impact of modern farm systems on farm animal well-being nor even to allege that such systems are inhumane. The "fairness" of the allegation depends on the definition of inhumane and the quality of the supporting data and argument. In fact, there is a wealth of data indicating that close confinement compromises the well-being of poultry, pigs and other farm animals. The questions now focus on the extent to which well-being is compromised, and the potential to develop appropriate confinement systems that retain most of the advantages for the farmers and the animals while eliminating most of the adverse effects.

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Promotion in Italy

SIR — I was very surprised to see that Baccarini *et al.* (*Nature* **356**, 188; 1992) write that "as regards clinical teaching in haematology, the activity... needs to be evaluated also in terms of clinical and professional ability..." The Italian law states in Article 41, law 380, that "promotion to full professor is by national public competitions in order to establish the scientific maturity of the candidates". As there are no written or oral examinations and no practical tests, how can their professional clinical capacity be evaluated?

The only element remaining of this law is the scientific activity of the candidates represented by their publications. If these are clinical papers they should obviously be evaluated as applicants for a clinical competition.

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Hadley protests

SIR — A recent story by Christopher Anderson concerning my dealings with Congressman John Dingell's oversight and investigations subcommittee of the US House of Representatives Committee on Energy and Commerce quoted a statement by the National Institutes of Health (NIH) concerning this matter (*Nature* **355**, 98; 1992).

The NIH statement insinuates that I have conducted myself improperly with respect to the subcommittee. This is patent nonsense. Moreover, contrary to the NIH statement, I have *not* "recently been reminded" about any NIH policy with respect to responding to congressional requests for information, nor is there any reason I should have been so reminded. NIH acknowledge their awareness that I was to be interviewed by subcommittee staff during the meeting reported in the *Nature* story, yet the NIH statement gratuitously asserts that it is NIH policy that employees must not "reveal or discuss without authorization confidential information obtained in the course of their current or previous duties." NIH do not suggest that their putative policy applies to requests for information from duly authorized investigative subcommittees of the United States Congress. The spurious raising of the "confidentiality" issue in this context is a red herring, an apparent response by the NIH administration to its demonstrated inadequacies, as indicated by Anderson's story.

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Statistics of chronic disease control

Richard Peto

Control of the chronic diseases of middle age is increasingly important, in developed and in developing countries, as other causes of premature death decrease. It requires bigger clinical trials, and better epidemiology.

DURING this century war, famine and pestilence have caused hundreds of millions of premature deaths, and we are now in the middle of rapidly growing epidemics of death from the AIDS virus and the cigarette¹. But, paradoxically, greater progress has been made this century than any other in the worldwide prevention of premature death, with large decreases in childhood mortality (and hence large increases in life expectancy). Over the past 40 years, life expectancy has improved by 20 years in the less developed regions and by 10 years in the developed regions of the world (see table). Although the current rate of improvement is greater in less developed regions, the process has gone further in developed countries such as Britain, where 100 years ago half the infants would expect to die before middle age and three-quarters would do so before 70, but where only 3% now expect to die before 40, still almost 30% would do so before 70. Thus, in Britain death before middle age has been largely avoided but death in middle age is still common, though it is largely avoidable: 80% of such deaths involve vascular or neoplastic diseases, for many of which better treatments and more effective preventive measures can be foreseen. Rapid progress is being made both in basic biological science and in applied laboratory studies of the nature, treatment and causes of such chronic diseases. But this progress needs to be

complemented by more reliable clinical trials of their treatment and better epidemiological studies of their causes.

Mega-trials

Large improvements in the curability of vascular or neoplastic diseases are notoriously elusive, but even moderate-sized reductions in a common cause of death may be worthwhile. Reliable clinical research into whether there is, or is not, a moderate difference between the effects of two treatments on long-term survival requires particularly strict control of

difficult in practice: very large numbers of patients must be randomized, perhaps in one or two 'mega-trials' or in an overview of several smaller trials.

It is only recently that randomized comparisons of different chronic disease treatments have become appropriately large. One of the most striking recent results has been from the ISIS-2 trial of acute heart attack², which showed that two common drugs, streptokinase and aspirin, could both produce a moderate but definite improvement in survival, avoiding about 5,000 deaths for every

FORTY-YEAR TRENDS IN MORTALITY (UN ESTIMATES)

	Infant mortality (per 1,000)			Life expectancy (years)		
	1950-55	1970-75	1990-95	1950-55	1970-75	1990-95
China	195	61	27	40.8	63.2	70.9
India	190	135	88	38.7	50.3	60.4
Africa	188	137	94	37.7	45.9	54.1
All 'less developed' regions	180	105	70	42.2	55.2	63.3
United Kingdom	28	17	8	69.2	72.0	76.1
United States	28	28	8	69.0	71.3	76.4
Former Soviet Union	73	26	20	64.1	68.6	71.3
All 'more developed' regions	56	22	12	66.0	71.1	74.9
World average	155	93	63	47.5	58.5	65.5

both systematic errors (biases) and random errors (the play of chance). Strict control of biases generally requires proper randomization and proper statistical analysis, without unduly data-dependent emphasis on the results in any particular subgroup (or on the results in any particular trial). Strict control of random errors is simple in principle, though

100,000 patients treated (Fig 1a). Before the 1980s streptokinase had been studied in dozens of trials that were too small, and this so confused the medical profession that the drug was rarely used. Then came two mega-trials^{2,3}, GISSI-1 and ISIS-2, and there was a transformation of cardiological practice⁴, saving thousands of lives a year.

An alternative to one or two large trials is a systematic overview (or meta-analysis) of dozens of smaller trials. In stage II breast cancer, for example, 'adjuvant' hormonal therapy for 100,000 women a year (Fig. 1b) achieves about 10,000 more 10-year survivors⁵. Early breast cancer is so common that such hormonal treatments could well save more lives than any other drug treatment for any type of cancer. Yet before the worldwide overview, they were generally thought to have been 'demonstrated' not to improve long-term survival. The Oxford Clinical Trial Service Unit has demonstrated strikingly definite results from various previously undervalued treatments⁶⁻⁹ that can, collectively, avoid a few more tens of thousands of premature deaths each year.

Better epidemiology

But, if millions of premature deaths a

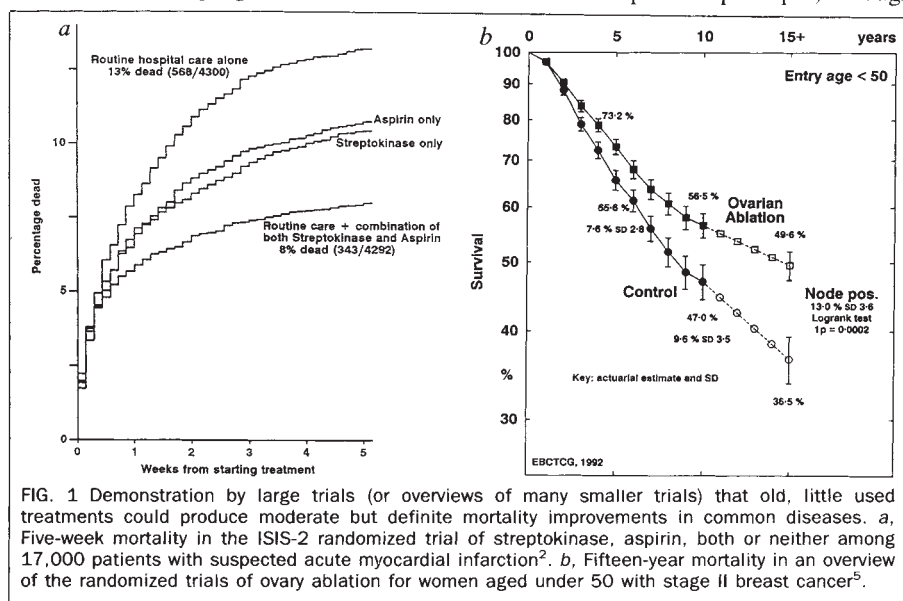


FIG. 1 Demonstration by large trials (or overviews of many smaller trials) that old, little used treatments could produce moderate but definite mortality improvements in common diseases. a, Five-week mortality in the ISIS-2 randomized trial of streptokinase, aspirin, both or neither among 17,000 patients with suspected acute myocardial infarction². b, Fifteen-year mortality in an overview of the randomized trials of ovary ablation for women aged under 50 with stage II breast cancer⁵.

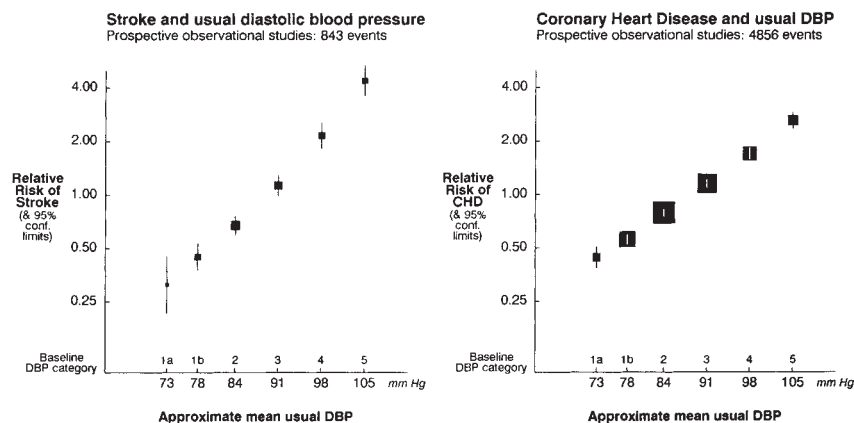


FIG. 2 Relevance of the usual diastolic blood pressure (DBP) to the subsequent rates of stroke or of coronary heart disease (CHD)¹¹. These prospective observational studies indicate that a long-term difference of 5–6-mm Hg in the usual DBP would correspond to about 35–40% less stroke and 20–25% less CHD. The randomized intervention trials have on average involved a difference of 5–6-mm Hg in blood pressure for an average of only 5 years, and in aggregate involved 38% (s.d. 4) less stroke and 16% (s.d. 4) less CHD, with similar-sized effects on fatal and on non-fatal events.

year in developed countries are to be prevented, then the effects of some of the really large causes of premature death, such as blood pressure, blood cholesterol and, particularly, tobacco must be controlled. All these effects have been seriously underestimated. Careful attention to the small statistical details makes a surprisingly big difference to the conclusions. Appropriate allowance for measurement errors (the 'regression dilution' effect) makes the real relationships with risk at least 50% steeper than generally supposed (Fig. 2)^{10,11}. When non-Western populations are considered, the relationships extend downwards¹⁰ to much lower risks of stroke and heart attack than is generally supposed (see Fig. 2 legend)^{12,13}. With the low blood pressure and low blood cholesterol levels still sometimes found in pre-agricultural societies, most strokes and heart attacks would be avoided. (The persistent media coverage of the

supposed hazards of cholesterol reduction has little scientific foundation, merely underlining the need for proper mega-trials of the large cholesterol reductions that 'statin' drugs can produce.)

For tobacco, the epidemic of premature death that is at last reaching its peak among the males in developed countries (Fig. 3) is now spreading to females in developed countries and to males in developing countries. Tobacco is the most important global cause of cancer, but it kills even more people by other diseases. During the 1990s tobacco is already causing about 3 million deaths a year, and on present smoking patterns it will probably be causing about 10 million deaths a year a few decades from now¹⁵.

Other major causes of chronic disease, although causing fewer deaths, may be easier to control. In China, for example, substantial reductions in the use of salt as a food preservative might prevent many premature deaths from blood-pressure-related disease¹⁶ and effective neonatal vaccination against hepatitis B virus¹⁷ could protect two or three million children in China a year against infection, about a million of whom would have died from chronic liver disease in adult life. Chronic lifelong infection with the hepatitis B virus, starting in early childhood, is the second most important reliably established cause of cancer in the world. But it can usually be prevented by the neonatal vaccination that should in 1992 be incorporated by the World Health Organization into the worldwide routine Extended Programme on Immunization.

Infective and chronic disease

Although the growing importance of chronic disease control has been stressed, the chief global health priority in the 1990s remains control of the main infective diseases. In 1980 there were

about 50 million deaths in the world, half in people under 45 years of age. Indeed, 15 million were under 5: in early childhood about 4 million deaths a year were due to diarrhoea, about 4 million to acute respiratory infections, and about 4 million to diseases that can largely be prevented by vaccination.

Since 1980, childhood vaccination rates have greatly increased, but so has smoking and, threatening to dominate world health next century, the AIDS epidemic is emerging. In the midst of so much preventable death from infectious disease, however, it is important to remember how much worse things used to be earlier this century. Although death rates from AIDS and tobacco are rising, those from most other big causes have been falling for decades. Global life expectancy is better now than life expectancy in developed countries earlier this century, and may next century become better than in developed countries today. But, to achieve this, we need to take seriously all the main avoidable causes of premature death, including infective and chronic diseases. Of the five or six thousand million people alive today, most have already survived the hazards of the first 5 years of life, and far more will eventually die of chronic disease in middle age (35–69) than will die from infection before then. Indeed, unless AIDS (or other new infection) completely transforms current patterns of premature death, even among those born this year about as many may die from chronic disease in middle age as die before middle age from infection. So, although easy control of infective disease remains the most important priority, the more successful it is the more people will survive to middle age, and the more important will become the control of the large avoidable causes of premature death from chronic disease, such as hepatitis B, blood pressure, blood cholesterol and, particularly, tobacco¹⁵.

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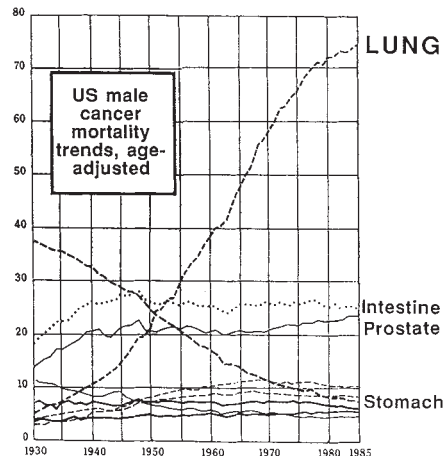


FIG. 3 Trends in US male cancer death certification rates, 1930–85, showing a large increase during 1945–85 in the lung cancer death rates¹⁴. (Nonsmoker rate is 5 per 10⁵; main increase in US male cigarette smoking was during 1915–45.)

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Electronic journals have a future

A conference on changing patterns of journal publication suggests that electronic journals may already have arrived, but that their management remains a problem for the future.

Chicago

EVERYBODY agrees that the world's journals are in flux, but nobody is sure what the future holds — or even when it will arrive. That was the general drift of a conference on "The role of journals in scholarly communication", held last week as part of the centenary celebration of the University of Chicago, still recognizably distinctive for its interdisciplinarity, for the intellectual daring of its faculty and for the unobtrusiveness of its administration.

What follows is not so much a formal report of the proceedings (which would be professionally improper for a participant) as an account of some of the issues raised by people who edit journals, who are responsible for buying them (librarians), who are sociologists of science or who are otherwise students of the phenomenon of the recent growth of the literature of scholarship. But that phenomenon defies accurate census.

For one thing, it is easier to count new titles than to record those that disappear. More important, the division between scholarship and journalism is not always sharp: Addison's *Spectator* rated several mentions, as did the *New York Review of Books* and the *Times Literary Supplement*. But nobody carried a torch for *Scientific American* or *La Recherche*, leaving unanswered the question of the role of good popularization in communication among scientists. But Ann Okerson from the Association of Research Libraries in Washington, DC, noted that 29,000 new serial titles appeared between 1978 and 1987, and estimated that the total extant is close to 250,000. (She also estimates that a year's supply of the journals indexed by the *Medline* database would be twice as high as the Washington Monument.

What drives this huge and expensive growth? Even accepting that scholarship has grown enormously, and the common railings last week at duplicate publication and the serial publication of breathless interim reports on progress ('salami slices'), the phenomenon calls for an explanation. The common thread in most explanations is that scholarly publishing has become a kind of vanity press, existing to serve the interests of its contributors. And why is that interest so clamant? That unpublished scholarship might as well never have been done is a principle generally accepted, but the use of published works, perhaps even weighted by a citation index, has also become a standard influence in academic appointments and promotions.

Budget-conscious librarians last week

complained not merely at publishers (who have increased the average prices of journals three times more quickly, in the past few years, than ordinary retail prices), but at their academic colleagues, who are given to insisting on subscriptions to journals in which they have published, however untried and expensive. The irony is that their problems (and those of journals) would be simplified if only the link between publication and promotion could be broken or made less tyrannical. But scholarship seems not yet ready to contemplate solutions.

That is one reason why electronic communications were given a generous green light last week. (Another is that electronic journals offer facilities denied the users of ink-on-paper journals, the facility to call up from within a text being read the text of another referred to, for example). Other conventional journals are seeking to meet librarians' anxieties about storage costs by exploiting the compactness of the compact disk, and would be helped by accepted standards. But the notion that full-blown electronic journals will soon be multiplying was taken for granted, although the question of just when that will be is still open.

Dr Edward Huth, for 19 years a distinguished editor of the *Journal of Internal Medicine*, is about to find out. He is the first editor of the electronic journal the American Association for the Advancement of Science plans to launch in July. The journal will carry reports of clinical trials with new drugs and treatments. Huth's enthusiasm for the project only partly derives from his ambition to get rid of the bias in conventional journals against reports showing clinical innovations to be ineffective. It will also be a proving ground for a new technology, a source of information about the economics of the operation and a way of assessing what the end-users really want.

That will be a formal journal, with every article put out rigorously refereed. By Okerson's account, informal electronic publishing is already streets ahead. The innovators are the physicists, whose use of academic computer networks for the distribution of preprints is a fact of life. She quoted without evident disapproval one physicist's declaration that the "technological issues are already settled, . . . the demise of certain journals has been under way for more than a decade, . . . we have learned to determine by title and abstract whether we wish to read a paper, . . . the small amount of filtering provided by refereed journals plays no effective role in our research".

There was not much dissent from Okerson's opinion that this trend will continue. Indeed, she notes that usage of the US network Internet is growing at 15 per cent a month, that an estimated 5 million people worldwide have access to some interconnected network (of which there are an estimated 7,500) and that a system in which anonymous reviewers' comments are replaced by the comments of identifiable critics and held with the same records might even be an improvement of the literature. And, the argument continues, much paper would be saved.

Even before that usage is general, the concept of the journal as an integrated whole may have withered away. Okerson quoted a chilling figure: of the 1.5 million items supplied to each other by her member-libraries last year, an estimated two-thirds consisted of single copies of articles from journals. In short, there is already a brisk trade in the components of journals rather than in the journals themselves. When the electronic age dawns, people will routinely be searching the networks for single research articles, raising awkward questions about the ownership of copyright.

There are other awkward questions, not fully discussed last week. One is the degree to which electronic publication within the research networks will satisfy individuals' wishes that the importance of their work should be recognized not merely by their fellow network users, but by their masters. If the research community is not ready to cut the link between promotion and publication, perhaps the sociologists of science should find out.

There is an even darker question, arising from the circumstance that the electronic networks as they are cost academic users (and even, sometimes, their institutions) nothing. What will happen if the governments at present meeting the costs of networks start making realistic charges for their use? Or if they make discriminatory charges against, say, foreign access? Or even have views about the nature of the material made available? Such developments, unlikely though they may be, could seriously damage the health of the research community.

Nature is for the time being an observer, not a participant, in these affairs. Issues of access from out-of-the-way places argue for it to remain an ink-on-paper journal. But even journals such as this might usefully have electronic antecedents at some stage. Last week's enthusiasm argues for sooner, not later.

John Maddox

PrP and the scrapie agent

Bruce Chesebro

WHAT is the relationship between the protein designated PrP (ref. 1) and the as yet uncharacterized infectious agents responsible for transmissible spongiform encephalopathies² such as scrapie? No one yet knows, but two reports in this issue point to ways in which the question may possibly be resolved.

Xi *et al.* (page 598³) show that the antibiotic amphotericin B can retard both the clinical symptoms and the appearance of protease-resistant PrP (PrP^{Sc}) aggregates in diseased brain tissue without affecting the levels of agent replication; this finding implies that PrP^{Sc} is not the infectious agent itself. Büeler *et al.* (page 577⁴) report that mice engineered to lack the gene for PrP show no detectable abnormalities in behaviour or development up to at least seven months of age. These results open the possibility of testing the involvement of PrP in both disease pathogenesis and agent replication *in vivo*.

Properties

The high resistance to inactivation of scrapie infectivity by chemicals or X-irradiation has led to suggestions that the agent might possess unique biophysical properties, and perhaps not even have a conventional nucleic acid genome⁵. Others favour the possibility that the transmissible agent is a conventional virus⁶ or an unusual combination of nucleic acid and protein^{7,8}. Scrapie-associated PrP^{Sc}, a macromolecular proteinase K-resistant protein aggregate derived from endogenous PrP^C, co-purifies with scrapie infectivity, which has given rise to debate over the possibility that PrP^{Sc} might itself be the transmissible agent. PrP^{Sc} is so far distinguished from PrP^C by its larger size and its relative resistance to digestion by proteinase K. Biosynthetic studies *in vitro* indicate that normal PrP^C, which is expressed on the surface of many cell types, is the precursor of PrP^{Sc}. The conversion to PrP^{Sc} probably occurs at the plasma membrane or along the endocytic pathway to the lysosome⁹. Once formed, PrP^{Sc} persists in cells, and its accumulation appears to be responsible for the cell damage observed in brain tissue.

Xi *et al.* hypothesize that amphotericin B interferes with a component of the infectious agent responsible for the conversion from PrP^C to PrP^{Sc}. Surprisingly, retardation of PrP^{Sc} accumulation had no apparent effect on the titre of the infectious agent detectable in the same brain samples, meaning that PrP^{Sc} itself may not be the transmissible agent responsible for scrapie. One weakness of

these data is the difficulty in quantifying both the PrP^{Sc} and the titre of the infectious agent in brain samples. This criticism has also been applied to experiments, promoting the opposite viewpoint, which showed a correlation between the inactivation of scrapie infectivity and the destruction of PrP^{Sc} during proteolysis *in vitro*^{1,10}.

Xi *et al.* also found that amphotericin B was effective only in hamsters infected with the 263K strain of scrapie. No effect was observed in mice or hamsters infected with two other scrapie strains. The reason for this strain-specific difference is unclear, but it might be explained if these strains could replicate in different types of brain cell. The results point to the desirability of studying different scrapie strains in attempting to understand the nature of these agents and their pathogenic mechanisms.

Several studies with transgenic mice have indicated that PrP plays a leading part in susceptibility to scrapie. Unlike normal mice, transgenic mice expressing hamster PrP are highly susceptible to hamster-adapted scrapie strains¹¹. Paradoxically, in other experiments, mice expressing a foreign mouse PrP genotype, which is usually associated with slower progression to disease, developed disease more rapidly¹²; in this case, the level and location of PrP expression may have been more important than the PrP genotype in influencing the tempo of disease.

Lastly, transgenic mice expressing a PrP gene with a specific mutation found in some cases of a familial human spongiform encephalopathy (Gerstmann-Sträussler-Scheinker syndrome) developed a degenerative brain disease¹³. These results might suggest that the mutation of PrP created a transmissible scrapie agent *de novo*. Alternatively, it could be that the PrP mutation increased the susceptibility of these mice to an unknown but ubiquitous 'conventional' virus. However, this disease differed from scrapie and all other related transmissible spongiform encephalopathies in that no protease-resistant PrP^{Sc} was detectable in the brains of afflicted mice. Furthermore, the disease does not seem to be easily transmissible. So it seems most likely that this model is not identical to scrapie, but is a manifestation of a metabolic disease due to expression of a mutant protein.

Although all of these transgenic mice models provide evidence that PrP is a susceptibility factor for the development of scrapie, none of them gives any evidence as to the nature of the agent itself.

Therein lies the importance of the report by Büeler *et al.*⁴ — their development of a viable normal mouse strain without a functional PrP gene may provide a unique opportunity to examine the involvement of PrP in replication of the scrapie agent. Tests of the susceptibility of these mice to inoculation with the agent are probably already in progress, and they should produce interesting results no matter what their outcome.

Possibilities

One can envisage at least three possibilities. First, there might be no clinical disease and no agent replication. This would show that PrP is required for both processes, and would suggest that PrP might either be a component of the agent itself or a receptor or other type of factor required for the agent to grow successfully in cells. Second, there might be successful agent replication but no apparent disease. This would support the current evidence that accumulation of aggregated PrP^{Sc} may cause destruction of brain tissue in a manner similar to other amyloidoses, but would imply that PrP^{Sc} is not an essential component of the transmissible agent. Third, there might be both disease and agent replication. This outcome seems unlikely in view of the evidence indicating a central role for PrP. However, Büeler *et al.* suggest that PrP function in normal mice might be redundant and replaceable by another protein in PrP-negative mice; perhaps the role of PrP in susceptibility to scrapie might also be assumed by another protein.

Taken together, the issues surrounding the spongiform encephalopathies remain complicated, perplexing and (not least) controversial. But we should soon have some interesting data to help address the various questions that need answers. □

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Conveying that sinking feeling

Andrew Watson

THE great ocean conveyor is the name coined by Wallace Broecker for the massive circulation cell which transports surface water north through the Atlantic until, in the Labrador and Greenland-Norwegian seas, it becomes sufficiently dense to sink and begin the journey southward again. Western Europeans have reason to be grateful for this circulation, because the heat it transports keeps us warm in winter and saves us from the rigours of a Muscovite climate. Now Broecker and Tsung-Hung Peng show on page 587 of this issue¹ that the ocean conveyor (less picturesquely known as the Atlantic thermohaline circulation) also transports a substantial amount of carbon dioxide of atmospheric origin between Northern and Southern Hemispheres. The implication is that, before the industrial revolution changed everything, the atmosphere must have transported an equal flux in the opposite direction, from south to north. Carbon dioxide concentrations must therefore have once been higher in the Southern Hemisphere than in the north. This in turn bears upon the current controversy over the size and location of the sinks on land or at sea for fossil-fuel CO₂.

Broecker is an acknowledged grand master of chemical oceanography, and his latest paper with Peng demonstrates well his skill at re-interpreting oceanographic data. Starting with widely available measurements on the distribution in the sea of phosphate nutrient and alkalinity (the excess of positive charge balanced by carbonate and bicarbonate ions), they perform simple manipulations to take account of the known effects other than exchange with the atmosphere which would be expected to alter the carbon content. They thereby produce estimates of the concentration of carbon that the water would have contained had it been isolated from the atmosphere. The difference between this 'expected' carbon content and what is actually measured is the amount that has been taken up from, or lost to, the atmosphere. Another quick correction for the amount taken up since the industrial revolution and they arrive at estimates for the pre-industrial ocean surface uptake.

The authors' results show a large excess of CO₂ of atmospheric origin in the North Atlantic compared with the Antarctic. Multiplying the difference by the water flux, the rate at which the conveyor turns, they obtain the flux of carbon, 0.6 gigatonnes (Gt) a year, which the Atlantic transported from north to south before 1850. It all sounds

so reasonable that one wonders why it was not done a decade ago.

The north-south gradient of atmospheric CO₂ has therefore changed sign during recent times. Whereas previously CO₂ concentrations were higher in the south owing to this ocean transport, the north now has the higher values and the gradient appears still to be increasing^{2,3}. Broecker and Peng also say that their result eliminates the need to challenge the earlier estimates of the magnitude of the ocean uptake and the need to postulate a large mid-latitude sink on the land surface, but the reasoning behind this statement is rather more obscure.

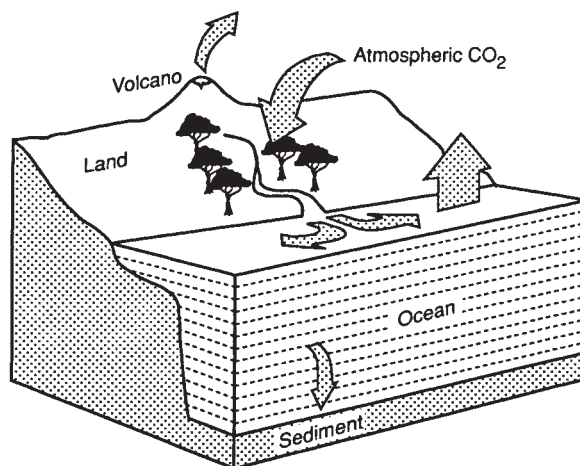
Until 1990, the accepted view was that the oceans were the main natural sink for anthropogenic CO₂ (the excess produced by fossil-fuel burning and tropical

deforestation). Ocean uptake was thought to be about 2 Gt of carbon per year, a figure obtained by 'box-diffusion' analyses calibrated against observations of the uptake of ¹⁴C generated in the atmosphere by nuclear testing^{4,5}. A more detailed ocean general circulation model has recently given essentially the same result⁶.

Confidence in the models was severely shaken with the publication in 1990 of a study by Pieter Tans and colleagues⁷. This combined an analysis of the distribution of CO₂ in the atmosphere with the first detailed attempt to calculate fluxes into the sea using measurements of sea-surface CO₂. Tans *et al.* found that the observed small north-south gradient of CO₂ in the atmosphere pointed to a natural sink in the Northern Hemisphere which must exceed that in the south by about 2 Gt yr⁻¹, while the total sink must add up to at least 2.3 and more likely 3.5 Gt yr⁻¹ (depending on the size of the poorly known deforesta-

Balancing the carbon budget

IN related work, Sarmiento and Sundquist point out on page 589 of this issue¹¹ that estimates of global air-sea CO₂ fluxes based on tracer-calibrated models of ocean mixing are not directly comparable with the air-sea flux calculations performed by Tans *et al.*⁷. The models estimate only the additional CO₂ now being taken up as a result of the additional atmospheric concentration due to industrialization. By contrast, the air-sea exchange approach refers to the actual present-day flux. The two calculations would be expected to agree only if, pre-industrially, the net



The pre-industrial steady-state carbon cycle: to balance the flux of carbon coming down rivers, there must have been a CO₂ flux of the order of 0.5 gigatonnes of carbon per year from ocean to atmosphere and from atmosphere to land. Volcanic activity and sedimentation fluxes provide smaller net inputs and outputs to the system.

transfer from air to sea was zero. But in fact, at that time there must have been a net flux from the oceans to the atmosphere. This is evident because rivers transport a substantial amount of carbon (in the form of organics and bicarbonate) from land to sea. Most of this carbon has come rather directly from the atmosphere or the land biota, and therefore a roughly equivalent flux must be postulated from the sea to the air to complete the steady state. After making corrections to this simple picture to account for carbon lost to sediments or produced by volcanic and metamorphic activity, Sarmiento and Sundquist arrive at a figure of 0.4 – 0.7 gigatonnes of carbon per year as the probable pre-industrial flux of CO₂ from the oceans to the atmosphere. This is the amount that absorption calculated from a tracer-calibrated ocean model might be expected to exceed the true present-day air-to-sea flux. In their 'new budget', Sarmiento and Sundquist also include a rough estimate of another correction, concerned with the ocean surface 'skin-temperature deviation', which raises the CO₂ concentration at the interface (and which they kindly attribute to me). Robertson and I have recently completed an accurate evaluation of this effect¹², and the results show that it too helps substantially to narrow the gap between the two methods of arriving at the ocean uptake of CO₂.

A.W.

tion source). Others had come to similar conclusions^{8,9}, but the new element introduced by Tans *et al.* was a calculation of the oceanic sink north of 15° N, which their sea-surface data indicated to be at most 0.6 Gt yr⁻¹. Because the northern sink was not predominantly in the ocean, they concluded that it must be on land, and that the principal global CO₂ sink (at least 2 and up to 3.4 Gt yr⁻¹) is associated with the vegetation of the Northern Hemisphere continents. Of several models they tested, none having a total ocean sink greater than 0.8 Gt yr⁻¹ was consistent with the data.

Broecker and Peng's work indicates that, before the industrial revolution, the North Atlantic was taking up 0.6 Gt yr⁻¹. Today, therefore, it must surely be taking up more than this, as the constantly rising atmospheric level will tend to drive more CO₂ into the surface water. It therefore seems very likely that Tans *et al.*, who obtained at most 0.6 Gt yr⁻¹ for the entire Northern Hemisphere oceans, underestimated the northern ocean sink and therefore had to assume an unrealistically large vegetation sink to balance the budget. For example, suppose that the difference between the Northern and Southern Hemisphere ocean sinks (call them *N* and *S*) remains the same today as it was pre-industrially, then (*N*−*S*) = 1.2 Gt yr⁻¹ by Broecker and Peng's analysis. If the total ocean sink (*N*+*S*) is 1.8 Gt yr⁻¹ as suggested by the tracer-calibrated models of the ocean sink, then simple maths gives *N*=1.5 and *S*=0.3 Gt yr⁻¹ for their present values. An additional contribution from land vegetation in the north of 'only' about 0.8 Gt yr⁻¹ is then required to satisfy the requirement that the total (land plus ocean) northern sink exceeds that in the south by about 2 Gt yr⁻¹.

So is the problem solved? Well, no. This kind of picture requires that the estimate by Tans *et al.* of the Northern Hemisphere's ocean sink was too low by a factor of at least 2.5. While the cover-

age of the present surface CO₂ database leaves much to be desired even in the better-studied northern oceans¹⁰, an error of this size seems to point to a systematic problem with either these data or the air-sea exchange formula used for the calculation. The jigsaw still therefore has pieces missing — the paper by Sarmiento and colleagues, also in this issue¹¹ is almost certainly one of them

(see box) — but at least it is becoming clearer from which part of the overall picture those pieces come. We can be confident that the gap between the different methods of calculating the ocean sink will soon narrow. □

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NEUROBIOLOGY

From cell line to brain

Joshua R. Sanes and Jeff W. Lichtman

In terms of the diversity of cell types it contains, the vertebrate central nervous system is unrivalled. Even within any small region, a great variety of neurons and supporting cells co-exist, their functions and morphologies completely integrated. How this cellular diversity is generated, and how the individual cells interact to form functional units, are central questions of neurobiology. Two groups^{1,2} have reapplied a classical embryological technique to these issues — transplantation of developing cells from one site or animal to another, and analysis of the fates they adopt. The new wrinkle is the use of neural cell lines instead of primary neural cells. The results offer glimpses of the remarkable power of the developmental milieu to transform uncommitted blast cells into highly differentiated neurons and glia, and make them part of the whole.

It has long been appreciated that cells from developing neural tissues can adopt new fates when transplanted from one region of the developing nervous system to another. For example, neural crest cells can be grafted from one level of the neuraxis to another, whereupon they differentiate into all the crest derivatives appropriate to the host site³. More recently, cells from cerebral cortex have been transplanted from one individual to another, and some have acquired properties characteristic of the site⁴ or developmental stage⁵ of the host rather than of the donor. These and other experiments⁶ support the idea that many neuronal phenotypic choices are determined by extrinsic cues specific to a particular region.

In the new work, the two groups modified this approach in similar ways. They cultured mouse brain cells (Renzan *et al.*¹ from hippocampus, Snyder *et al.*² from cerebellum) at a stage when progenitors were still dividing, and immortalized them by infection with a replication-defective retrovirus bearing an oncogene. Following establishment of clonal lines, cells were marked with one of several labels (³H-thymidine, a transgene or phagocytized fluorescent beads)

and reinjected, more-or-less isotopically and isochronically (that is, into neonatal hippocampus and cerebellum, respectively). Weeks to months later, the brains were sectioned and the engrafted cells identified.

Both groups report the same principal results. First, the implanted cells survived and proliferated, but the proliferation was limited and apparently controlled. Second, the cells differentiated into recognizable neurons and glia, as characterized by light and electron microscopy. Third, the phenotypes were appropriate for the host site — for example, dentate granule cells in hippocampus (see figure), and internal granule and basket cells in cerebellum. Finally, the cells integrated beautifully into the host architecture — the neurons, for instance, sent out axons along appropriate pathways¹ and formed synapses with endogenous partners². Thus the immortalized, implanted cells participated in brain development about as well as their endogenous neighbours.

This approach will benefit at least two groups of investigators. Developmental neurobiologists will want to use such cell lines as vehicles to ask how the relevant genes regulate and are regulated by differentiation. Genes can be introduced by transfection or infection, and their effects assessed in implanted cells and in their neighbours. It is a good bet that many important genes have effects *in vivo* that are not readily predictable from their activities *in vitro*, and this can now be studied directly. Similarly, regulation of endogenous or transfected gene expression can be assessed in the implanted cells, using cytochemical stains for *in situ* detection of the gene products.

Researchers with clinical interests may also benefit, as they have been grafting a variety of cell types into lesioned brains in attempts to restore lost function and to deliver substances (for example, trophic factors) that might promote the survival of compromised endogenous cells⁷. Diseases for which such therapies are currently envisaged (and are, in a

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RÉSUMÉ

Beam me up

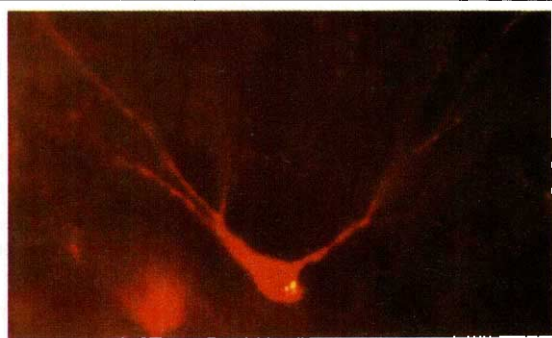
THE identification of C_{60} fullerene emerged from a search for interstellar carbon compounds. So it is appropriate that researchers at the Jet Propulsion Laboratory are now looking at the molecule as a fuel for ion-propulsion engines to be used in spacecraft and satellites (S. D. Leifer *et al.*, *J. Propulsion and Power*, submitted). Such engines, rather than harnessing the chemical power released in burning rocket fuel, would use the thrust from accelerated ion beams to drive spacecraft or steer satellites. Xenon is one favoured fuel for ion propulsion. But C_{60} has a mass (720 atomic units) 5.5 times larger, a projected ion-production cost less than half as large (90 electronvolts per ion), and an ionization cross-section 17.5 times larger ($90 \text{ \AA}^2$). It is also easily stored. Leifer *et al.* will shortly start experiments to measure the ionization properties of C_{60} under realistic conditions and investigation of its behaviour in a plasma discharge.

All spruced up

THE discovery of well-preserved stems of what seem to be conifers from Upper Carboniferous rocks in the West Midlands of England suggests that the origins of conifers must be sought somewhere in the middle of the period. The structures, which are some 300 million years old, were preserved in exquisite detail by a volcanic ashfall. They have key conifer characteristics, although they are hard to tell apart from stems of the extinct and possibly related cordaites (J. Galtier *et al.*, *Proc. R. Soc. Lond. B* **247**, 211–214; 1992). Nevertheless, the stems complement an earlier find of fossilized leafy conifer twigs from slightly older rocks, thought to be the earliest-known record of conifers in the fossil record.

On high

THE amphetamine derivative MDMA — familiar to acid-house freaks as ecstasy — may owe both its behavioural and toxic effects to a high selectivity for 5-hydroxytryptamine (5-HT) transporter proteins (G. Rudnick and S. Wall, *Proc. natn. Acad. Sci. U.S.A.* **89**, 1817–1821; 1992). The bizarre tactile enhancement experienced by devotees is not produced by amphetamine itself, which is more selective for catecholamine transporters. MDMA causes release of 5-HT which correlates with the morphological extent of neurotoxic damage. Rudnick and Wall show that MDMA stimulates 5-HT efflux through plasma-membrane and vesicular transporters responsible for its uptake and storage in nerve terminals. Its high potency and stereospecificity correlate with pharmacological data from behavioural studies, suggesting that this interaction may be why the drug is abused.



High-power photomicrographs of individual hippocampal stem cells 12 days after implantation into the dentate gyrus granule cell-layer of a young rat. The cells have started to differentiate in ways characteristic of the host tissue, showing arborization into the molecular layer and a single process extending down into the hilar region. (Photographs from ref. 1 courtesy of Miles Cunningham.)



few cases, being tested) range from Alzheimer's to Parkinson's to epilepsy. Because it is difficult to obtain primary cells in large numbers, and because such cells are notoriously difficult to render transgenic, the search for alternatives has been intense. Here, too, the new cell lines, and others like them, may prove useful.

What do the results of Renfranz *et al.* and Snyder *et al.* tell us about the interplay of the intrinsic predilections and intercellular signals that regulate phenotypic choice in the brain? Observations such as these are often taken to support an 'instructive' model: that the engrafted cells are initially plastic, and that their fate is determined by factors present in the host. In fact, however, isotopic and isochronic grafts do not rule out the alternative possibility that the cells are already programmed, and that the host environment is merely permissive. In an instructive model, the hippocampus and cerebellum (which are unusual in bearing large populations of neurons that are generated in early postnatal life) might contain a developmentally regulated series of factors that first stimulate the proliferation of neuroblasts, then cause them to withdraw from mitosis, and finally induce their differentiation. Alternatively, however, this entire sequence may have been programmed into the engrafted cells, requiring only the appropriate trigger

from the host. Analysis of heterochronic grafts — that is, grafts into earlier or later host developmental stages — might distinguish between these alternatives.

A similar dichotomy applies to the regional specificity of differentiation. In isotopic grafts, the appropriateness of the phenotypes could reflect environmental instruction of a multipotential progenitor or expression of a limited repertoire in a permissive environment. Here, however, Renfranz *et al.*¹ have already performed the crucial experiment. They implanted cells of their hippocampal line into the cerebellum, and showed that the engrafted cells survive, proliferate moderately and differentiate. Strikingly, the implanted cells look not at all like hippocampal cells, but acquire unmistakable morphologies of cerebellar neurons (granule and basket cells) and glia (Bergmann glia). The inescapable conclusions are that a single

progenitor can produce both telencephalic (hippocampal) and rhombencephalic (cerebellar) cell types; that the environment determines these phenotypic choices; and that cells introduced midway through development can nonetheless become part of a seamless whole.

Given the large body of previous work showing the remarkable plasticity of neural development^{2–7}, the importance of the new studies may rest less on the results themselves than on the methods used to obtain them. As in conventional transplantation experiments, the observation of regulative ability could reveal more about cells' potential under unusual circumstances than about their actual fate in normal development. But the substitution of manipulable, homogeneous cell lines for hard-to-handle, heterogeneous fetal cells should enable penetrating analyses of the signals and signal transducers that determine cell fate in the developing brain. □

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A boost for high flyers

Gary Zank

HIGH-ENERGY cosmic rays which bombard our atmosphere have been accelerated to their high velocities by collisions with irregular magnetic fields in our Galaxy, Bryant *et al.* show on page 582 of this issue¹. With their sophisticated calculations, the authors show that the mechanism, first proposed by Fermi² in 1949, can account for the remarkably high energies of the most energetic cosmic rays.

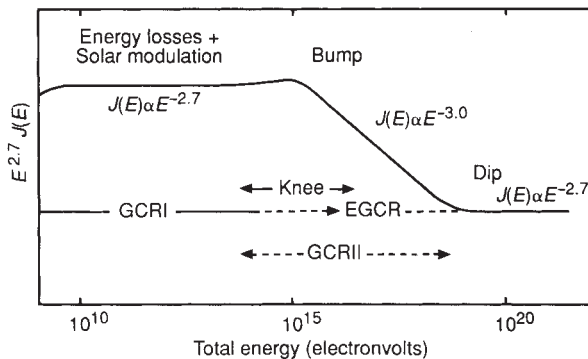
Thought at first to be γ -rays, cosmic rays are in fact composed predominantly of positively charged, highly energetic particles such as accelerated nuclei. The modern idea that cosmic rays are associated with supernova remnants (the turbulent debris of exploded stars) dates back to a brilliant paper by Baade and Zwicky³ in 1934, but the first compelling theoretical explanation was that advanced by Fermi. Fermi argued that particles are energized through head-on collisions with moving magnetic 'mirrors' in the interstellar medium, the energy gained being proportional to their initial energy. Fermi imagined these mirrors would be associated with slowly moving molecular clouds which traverse the Galaxy, but the rapidly expanding shock fronts of supernova remnants are now considered a more likely site. The particles' resulting energy (E) distribution (assuming their escape from the acceleration region does not depend on their energy) should be a power law whose (negative) exponent is the ratio of the particle acceleration timescale and the escape timescale: $N \propto E^{-T_{\text{acc}}/T_{\text{esc}}}$.

Fermi's arguments, and subsequent refinements, are both compelling and disturbing — compelling because of their simplicity and universality, but disturbing because the full underlying physical theory is embedded in the two parameters T_{acc} and T_{esc} . To account for the observed cosmic-ray spectrum, it is necessary for $T_{\text{acc}} \approx 1.7 T_{\text{esc}}$, although there is no good physical reason for this factor, nor even for why $T_{\text{acc}}/T_{\text{esc}}$ should be constant throughout the Galaxy. Fermi's argument is so general that it can almost be regarded as an interesting property of a certain kind of stochastic system independent of any particular theory.

Perhaps the most damning argument against Fermi's original formulation of the problem is that cosmic rays, being at most 10^6 – 10^7 years old (according to dating by ^{10}Be isotopes), have to be accelerated to their enormous energies

very rapidly. Indeed, because the characteristic acceleration time can be related to the speed u (10 – 40 km s^{-1} , for Fermi's molecular clouds) with which the magnetic mirrors move through the interstellar medium ($T_{\text{acc}} \propto (u/c)^2$, where c is the speed of light), there is simply not enough time for the mechanism to generate the most energetic cosmic rays.

Little further was done to improve



Schematic observed spectrum of galactic cosmic rays, in which the differential intensity $J(E)$ is multiplied by $E^{2.7}$ to emphasize the high-energy features (after Axford⁴).

Fermi's original idea (although considerable use has since been made of this approach in astrophysics) until the new work by Bryant *et al.*¹. These authors present a fully stochastic treatment of Fermi's original theory and argue, in particular, that the difficulty associated with the acceleration timescale is intrinsic to Fermi's development of the theory and results from his treating a stochastic process in a deterministic way. They argue that, rather than all particles gaining energy continuously at an average rate, the highest-energy particles do not gain energy in a "pedestrian manner", but that the acceleration process for these "high flyers" is much more efficient. Notably, Bryant *et al.* suggest that the Fermi theory may possibly account for particle energies up to 10^{18} – 10^{20} electronvolts (eV) per nucleon in the galactic disk.

Why should this be an important result? At present, the widely accepted physical theory for the acceleration of cosmic rays up to about 10^{13} – 10^{14} eV per nucleon is a fast or first-order Fermi mechanism in which particles are scattered back and forth across a shock wave in a supernova remnant by 'Alfvén' (magnetic) waves in the plasma, gaining energy at each cycle. Although the detailed plasma physics of the acceleration process awaits further elucidation, the theory is generally satisfactory and reproduces the observed spectrum, proportional to $E^{-2.7}$, very well up to 10^{13} eV (Axford terms⁴ this component galactic

cosmic ray I, GCRI). The particle acceleration time and short effective lifetime of supernova remnants (10^6 years), however, conspire to prevent particles gaining any energy above this cut-off. At the cut-off, something very curious happens to the spectrum (see figure). From 10^{13} to 10^{15} eV there is a slight bump, or knee, which is followed by a second power-law region (GCRII, with differential intensity proportional to E^{-3}); at 10^{18} eV, the spectrum dips and then returns to an $E^{-2.7}$ distribution.

The high-energy dip and flattening is not particularly remarkable and is explained easily in terms of two independent sources producing power law spectra (perhaps a combination of galactic and extragalactic, EGCR, populations, although the intensities should be fairly similar). However, to obtain the continuous convex feature at the knee by combining two independent spectra would require something akin to a "cosmic conspiracy" (to use Jokipii's colourful description⁵). It seems instead that the two spectra know of each other and that the GCRII component is obtained as a result of further acceleration of the GCRI

component. The smoothness of the fit indicates further that all GCRI particles in the vicinity of the supernova particle-accelerator cut-off energy must be allowed to participate in the post-acceleration process. The mechanism proposed by Bryant *et al.*¹ could re-accelerate the most energetic particles produced in supernova remnants up to energies of 10^{18} – 10^{20} eV, if there is a suitable distribution of scattering centres with large magnetic fields in the Galaxy.

Development beyond this level of understanding will require the direct determination of cosmic-ray composition and spectra up to about 10^{16} eV, which colleagues and I are now attempting⁶. If composition measurements show a gradual enrichment in heavy nuclei, then this might well be the signature of a multi-stage shock-acceleration process. On the other hand, an abrupt change in composition would signal the necessity for an entirely independent source and mechanism. □

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Parasitizing the cytokine system

Alan Sher

FOR a quarter of a century, the schistosome egg granuloma has been a textbook example of an immunologically based tissue response underlying infectious disease. The granuloma — a T-cell-dependent inflammatory reaction to ova deposited in host tissues by schistosome worms — was initially proposed to be a manifestation of delayed-type hypersensitivity¹. Years of immunological analysis have since produced a much more complicated picture of the egg granuloma as a dynamic orchestration of different T lymphocyte, cytokine and inflammatory cell responses, but this view must now be revised in the light of the observations of Amiri and colleagues reported on page 604 of this issue². Amiri *et al.* show that in the absence of T and B lymphocytes, a single cytokine, tumour necrosis factor α (TNF α), is sufficient to trigger egg-granuloma formation. Even more intriguing are their findings which imply that schistosomes use host TNF α as a reproductive stimulus.

Schistosomiasis, which is caused by schistosome worms and is otherwise known as bilharzia, is one of the most serious of the parasitic infections that afflict the developing world. Most attempts at immunological attack on the helminth have centred on a vaccine to prevent infection³. But egg deposition is the primary stimulus of pathology in humans, and the female reproductive cycle and egg-granuloma formation are important secondary targets for immunization strategies.

How do T lymphocytes trigger and co-ordinate the influx of such diverse cell types as macrophages, eosinophils, fibroblasts and giant cells into the granuloma? One approach to this question has been to identify the T-derived cytokines (lymphokines) associated with the host response to eggs and analyse their role by means of *in vivo* depletion experiments. From the initial characterization of the granuloma as a delayed-type hypersensitivity reaction requiring CD4⁺ lymphocytes, one would predict that lymphokines — interferon- γ and interleukin-2 (IL-2) — associated with the Th-1 subset of CD4⁺ cells⁴ would predominate in the response to eggs and egg antigens. Instead, the principal cytokines expressed at the time of peak granuloma formation in mice infected with *Schistosoma mansoni* are Th-2 products (IL-4 and IL-5)⁵ normally involved in the induction of immediate hypersensitivity and in providing help for B cells⁴. Indeed, host Th-1 cytokine secretion is partially suppressed by egg deposition⁵.

The biological significance of the Th-2

response to eggs has been confirmed in a study demonstrating impaired granuloma formation in mice treated with neutralizing monoclonal antibodies against IL-4⁶. But it is evident from other work with mice injected with IL-2⁷, anti-IL-2 antibodies⁸ or an egg-specific Th-1 clone⁹, that Th-1 cytokines may also influence granuloma formation, either directly or by promoting a subsequent Th-2 response⁸.

Amiri *et al.* now simplify this complicated picture by showing that a single T-cell-derived cytokine, TNF α , can trigger a granulomatous response. Severe combined immunodeficient (SCID) mice, which lack functional T and B lymphocytes, generate only minute cellular reactions to eggs deposited in the tissues by *S. mansoni*. The authors reconstituted granuloma formation in SCID animals by injecting them with syngeneic spleen cells from infected, immunocompetent mice. Surprisingly, systemic inoculation of supernatants from an antigen-stimulated Th-2 T-cell clone also restored the capacity of infected SCID mice to make granulomas. By means of antibody-depletion experiments, TNF α was identified

as the lymphokine responsible for the transfer of biological activity; indeed, infected SCID recipients of purified recombinant TNF α alone were found to produce granulomatous lesions only slightly smaller than those occurring in immunologically intact control mice not treated with the cytokine.

In vivo depletion of TNF α has been shown to prevent formation of granulomas elicited by *Mycobacterium bovis*¹⁰. What is unexpected about the new findings with *S. mansoni* is that systemically administered TNF α is in itself enough to stimulate the accumulation of many different cell types into a granulomatous lesion. Amiri *et al.* speculate that the pleiotropic stimulatory effects of TNF α on inflammatory cells, combined with the localizing influence of the egg and its released molecules, may account for the unique capacity of the cytokine to orchestrate granuloma formation. TNF α could also function by amplifying the production of mediators from non-T cells stimulated near to the ova.

A second provocative observation²

concerns the influence of TNF α on worm fecundity. Female schistosomes in SCID mice produced fewer eggs than did worms in normal animals. Egg output dramatically increased after *in vivo* injection of TNF α , as did fecundity *in vitro* after addition of the cytokine to explanted worms. It seems, then, that schistosomes may use host TNF α as a reproductive stimulus or embryogenic growth factor. The broader implication is that schistosomes 'parasitize' the cytokine network, employing T-cell-derived TNF α not only in their reproduction but also to envelope eggs in granulomas,

IMAGE
UNAVAILABLE
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REASONS

A cercaria of *Schistosoma mansoni*, the final larval stage which develops in an intermediate, snail host.

thereby protecting the host against toxins released by the ova.

These intriguing observations will undoubtedly stimulate a search for further examples of co-evolution of parasites and host cytokine responses. In the case of schistosomes, the hypotheses that

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female schistosomes possess parasite-derived TNF α receptors linked to a re-productive signal pathway, and that TNF α is the primary lymphokine regulating granuloma formation, can be readily tested. Because TNF α is notorious as a double-edged sword¹¹, its potentially harmful effects on both parasite and host must also be considered. So it is likely that in schistosome-infected hosts, TNF α production is itself tightly controlled. Down-regulatory cytokines such as IL-10 or TGF β , also produced in

schistosomiasis^{12,13}, may perform this function. Indeed, the general depression of immunopathology, immune responsiveness and egg production seen in the later stages of infection¹⁴ may in part reflect the need to tame the production of TNF α by T lymphocytes and other cells. □

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CRYSTALLOGRAPHY

Copernican revolution declared

A. L. Mackay

CRYSTALLOGRAPHIC symmetry theory has been unchanged for a long time and David Mermin argues¹ that a revolution is overdue. He and his colleagues at Cornell University, he suggests, have started one.

Mermin has shown that, following the discovery of icosahedral quasicrystals and their description in terms of a baroque six-dimensional framework, crystals and quasicrystals can be brought together into a single taxonomy by moving one's view point (just as Copernicus displaced the Earth-centred Ptolemaic cosmology with a Sun-centred one) to the description of symmetry in reciprocal space; that is, by concentrating on diffraction phenomena rather than on the positions of the atoms.

On this basis Mermin and his colleagues¹⁻⁴ have re-done all of symmetry theory, deriving the 230 space groups (a century after Fedorov, Barlow and Schoenflies first developed them) starting with relations between phases of the Fourier transform instead of with the real-space scaffolding of screw-axes and glide-planes. They have also rigorously determined the symmetry groups allowed for icosahedral quasicrystals within the same formalism.

Both crystals and quasicrystals give diffraction patterns with sharp spots, each spot corresponding to a sinusoidal density wave in real space. When considering a radio receiver we can either go outside and measure the aerial in real space, or stay indoors and work with the frequency scale on the set. Analogously, crystallographers and solid-state physicists look at crystals differently, the former seeing the atoms in real space and the latter preferring reciprocal space for describing crystal properties. Translation between them is often difficult.

Quasicrystals are highly ordered textures in which 5-, 8-, 10- or 12-fold symmetry appears in the diffraction patterns, these symmetries being disallowed in orthodox crystals. Their detailed

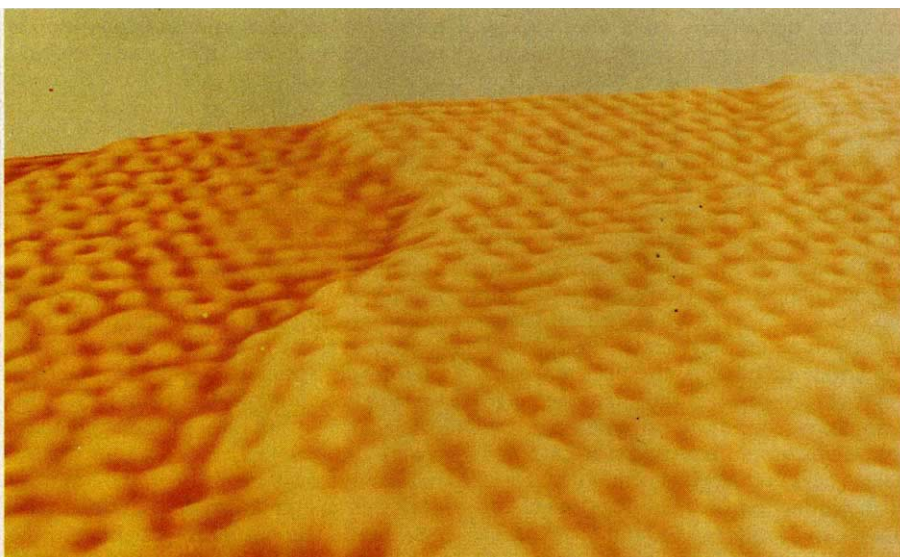
structure is still not clear although good models have been produced. The Penrose tiling pattern is an example of a structure which has the diffraction properties observed for quasicrystals, giving an indefinitely large number of spots, any pair separated by weaker ones.

The price we pay for X-ray crystal structure analysis is the assumption that the unit cells of a crystal are all the same. This enables us to confine our measurements of X-ray scattering to the lattice points of the reciprocal lattice. The intensity of a diffraction spot indicates the amplitude of a sinusoidal density wave in the crystal but the phase of this wave is lost in the recording process. Knowledge of the phase is essential if the real structure is to be recovered from the diffraction data. In modulated crystals where, for example, certain atoms may be displaced from the regular lattice by a sinusoidal modulation that is not one of the reciprocal lattice vectors, unit cells are not all the same and extra spots may appear in the diffraction pattern.

Quasicrystals can be regarded as having a special kind of modulation where the carrier wave has disappeared (or we cannot tell which frequencies are the carrier waves and which are sidebands).

Following Bienenstock and Ewald⁵, Jeffery⁶ and Wells⁷, Mermin and colleagues have derived the 230 classical crystallographic space groups in terms of the phase relationships between symmetry-equivalent reflections. Moreover, they have assimilated the theory of quasicrystals into this in one coherent scheme. Rather than the positioning of the actual atoms, reciprocal space, the spectrum of diffraction from the crystal (or quasicrystal), is taken as the primary domain and it is assumed that only the amplitudes of the diffracted radiation are available. This is usually the case for X-ray diffraction in crystallography, but experimental methods of determining the phases exist. These methods comprise isomorphous substitution of heavy atoms (the universal method in protein structure analysis), the 'direct methods' which earned Karle and Hauptman the Nobel prize for physics^{8,9} and electron microscopy. Other physical methods involving X-ray interferometry exist but are still impractical.

It is not news that there are 230 space groups and most crystallographers are content that they have been listed once and will not worry about a new derivation. Fortunately Mermin and his colleagues have not found any mistakes in the International Tables where the groups are listed, although, with the example of Frankenheim in mind, it was worth checking. (Frankenheim derived the Bravais lattices six years before Bravais but, as he made a mistake, duplicating one of the 14, they are called the 14 Bravais lattices and not the 15 Frankenheim lattices. Perhaps there should be



Now you see it — scanning tunnelling microscopes are making it possible to view quasicrystals directly. (Micrograph courtesy of A. R. Kortan.)

a Frankenheim medal for those who get a discovery almost right.)

Since Mermin assumes that direct observation is ruled out, he replaces 'identity' of structures with 'indistinguishability' on the basis of their diffraction patterns. By indistinguishability he means structures that give the same amplitudes in their diffraction patterns and which, consequently, have in real space the same Patterson functions, not only for pair-wise autocorrelations but for n -wise correlations. This concept of homomorphic structures — different structures giving the same diffracted amplitudes but different phases — was introduced into crystallography with the development of the Patterson function, but with no real examples. However, quasicrystals have this property, which in real space is "weaker than periodicity — that given any subregion, a translation can be found through a distance of the order of its dimensions, that takes the subregion into an identical one"¹.

In fact, Mermin has re-discovered structure invariants, relationships between the phases of the diffracted waves, and some of the other apparatus of the direct methods of Karle and Hauptman, but he restates them in the language of gauge theory. Perhaps Mermin can now extend direct methods to quasicrystals and the electron microscopy of amorphous materials. Phase relationships should apply all over the reciprocal lattice. The *a priori* knowledge that matter is made of atoms remains to be worked into Mermin's formulation and there is every indication that this is a powerful constraint (making Karle and Hauptman's methods possible).

With the steady improvement of transmission electron microscopy and associated image processing techniques, and especially with the appearance of the scanning tunnelling microscope and its variants, the arrangement of atoms can often be observed directly in real space. The understanding of real materials, such as feldspars, where interpretation of very complex diffraction patterns has been prohibitively difficult, has been revolutionized by direct imaging. Intergrowths of different composition, often amounting to complex modulation, can be seen immediately. Quasicrystal surfaces can also be imaged by scanning tunnelling microscopy (see figure), verifying the inferences from diffraction effects. Present-day crystallographic interest is firmly in real space where the players are the atoms.

Mermin's approach also rejects the six-dimensional description developed for icosahedral quasicrystals. He complains that it is unsatisfactory to regard a three-dimensional structure as a projection of four-dimensional space (as is done in the case of a simple modulated

crystal) or of six-dimensional space (in the case of icosahedral quasicrystals), as we would then have to ask what is the full structure of six-dimensional crystallography of which our own three-dimensional crystals are a special case. But indexing the faces of hexagonal crystals with four indices is an ancient practice of crystallographers and exhibits the symmetry better, although, as Frank¹⁰ has pointed out, it could be represented as a projection from four dimensions. Handling quasicrystals this way is just an extension of this practice.

The question is whether the reciprocal lattice approach can tell us anything new, as it is probably better for students to continue to begin more concretely with symmetry operations in real space than with phase relationships. Accordingly, it is not really a copernican revolution of viewpoint to reformulate the whole of space-group theory in terms of reciprocal space as Mermin suggests¹. Nor is it a simple derivation. (We should recall Arthur Koestler's finding¹¹ that

METEORITICS

Stardust memories

I. P. Wright

MINUTE grains of silicon carbide brought to Earth embedded in primitive meteorites turn out to be 'star dust', the condensed remains of stars that existed long before our own Solar System. As became apparent at a recent meeting on planetary sciences*, these grains carry not only the chemical signature of the pre-solar environment, but also force astronomers to consider some of the more esoteric nuclear burning processes that can occur in stars.

The silicon carbide grains are often even less than a micrometre across (see figure), and are found in primitive carbonaceous chondrite meteorites at the level of a few parts per million, equivalent to just 4×10^{-5} of the total silicon in these samples¹⁻³. They were discovered, in part, because of their extreme inertness towards chemical processing, being some of the only materials left after subjecting meteorites to the actions of various acids. It transpires³ that the grains pre-date the Solar System by 10–140 million years. Clearly materials of this nature are of immense importance to astrophysics, as they are amenable to chemical and isotopic analysis in the laboratory.

The salient features of SiC grains are as follows. They have a wide range of silicon and carbon isotope compositions, with accompanying nitrogen also being isotopically highly variable. The carbon

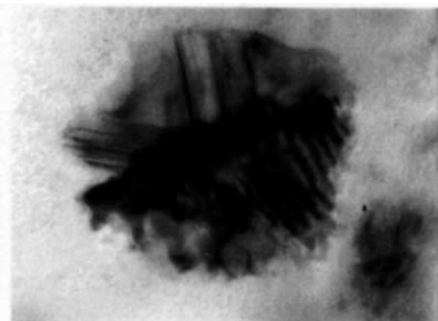
the 40 epicycles needed in the ptolemaic cosmology were replaced by 48 in the copernican system.) The real significance is that it clearly pays to work dialectically backwards and forwards between real space and reciprocal space and to keep trying to translate concepts in one language into the other. The dialogue between solid-state physicists and crystallographers is similarly valuable. □

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isotope ratio $^{12}\text{C}/^{13}\text{C}$ ranges from 2 to 2,500; it is 90 for the Solar System. The isotope compositions of elements such as xenon, krypton, barium, strontium and neodymium trapped in the grains reveal their origin in a neutron-rich environment in which the slow neutron-capture process (s-process) of nucleosynthesis can occur. Neon is enriched in the isotope ^{22}Ne . And the grains also reveal evidence⁴ that they once contained radioactive ^{26}Al , which decays to give ^{26}Mg .

These properties can be successfully explained by a model in which the grains condense in the envelope of an old low-mass carbon star which has reached the asymptotic-giant-branch (AGB) phase of evolution and is thermally pulsing⁵. In such a star, alternate hydrogen and helium burning outside its



A single SiC grain, 133 nm across, embedded in a matrix of microdiamonds, from the Indarch meteorite. (Micrograph courtesy of Martin R. Lee.)

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inert core leads to a source of neutrons (through the reaction $^{13}\text{C}(^4\text{He}, n)^{16}\text{O}$ in which ^{13}C fuses with a helium nucleus, releases a neutron and transmutes into ^{16}O), so allowing the s-process nucleosynthesis to take place.

One of the prime questions is whether a single star was involved or whether several stars contributed to give the wide range of isotope compositions. In models of thermally pulsing AGB stars, variations in carbon isotopes are readily explained, as consecutive episodes of hydrogen and helium burning result in, respectively, enrichments of ^{13}C and ^{12}C . As the components of these different events are mixed together, a variety of carbon isotope ratios can be produced. Thus, a single star can produce the range of carbon isotope compositions seen in the SiC grains. The relationship of silicon isotopes has also been explained in terms of evolution of a single star — original ^{28}Si -rich material is considered to become enriched in ^{29}Si and ^{30}Si as a result of neutron capture during the helium-burning phase⁶. But the observed relationship of $^{29}\text{Si}/^{28}\text{Si}$ and $^{30}\text{Si}/^{28}\text{Si}$ does not match that predicted from theoretical modelling of AGB stars.

New work reported by D. D. Clayton and L. E. Brown (Clemson University) shows that the silicon isotope composition in an AGB star can be radically affected by the neutron-capture reaction $^{33}\text{S}(n, ^4\text{He})^{30}\text{Si}$. At the same time, ^{22}Ne is turned into ^{25}Mg and ^{26}Mg by the helium-burning reactions $^{22}\text{Ne}(^4\text{He}, n)^{25}\text{Mg}$ and $^{22}\text{Ne}(^4\text{He}, \gamma)^{26}\text{Mg}$, where γ is a radiated γ -ray photon. When the neon is exhausted, the magnesium begins to burn thereby producing the lighter silicon isotopes. Eventually ^{25}Mg becomes exhausted, and the silicon isotope ratios begin to evolve to produce something like that seen in the SiC grains.

The model is flawed, as the effect on the silicon isotope ratios is 100-times that actually seen. What has to be inferred, if the model is correct, is that the isotopically enriched silicon, produced from helium burning, is mixed with normal silicon in the star's envelope and thus diluted. A consequence of this new model is that magnesium isotope ratios change quite drastically — incomplete magnesium burning may give rise to excesses in ^{26}Mg , which could subsequently be misinterpreted as the decay product of ^{26}Al .

The s-process isotope signatures observed previously in SiC have in some cases been obtained by making measurements on collections of grains (there simply not being enough atoms of the appropriate nuclides in individual crystals). The noble gases xenon and krypton, which provided the first unequivocal evidence that certain components in meteorites were formed through s-

process nucleosynthesis, are measured in this way; isotopic measurements of neon have also been obtained by lumping together large numbers of grains. In a new study, R. H. Nichols (Washington University) and colleagues have used a laser probe to liberate helium and neon from individual SiC grains (analysed previously for their Si and C isotopic compositions). This work shows that only 4 per cent of all SiC grains contain ^{22}Ne and ^4He — the rest are gas-poor. The ^4He and ^{22}Ne are characteristic of processing in an AGB star. What is of interest here is the observation that most grains appear to contain no trapped gases. If these were degassed at the time when the solar nebula spawned primitive meteorite parent bodies, then previous estimates of the ages of the grains (which are assessed by making measurements of neon isotopic compositions) could be erroneously low. As such, the ages of SiC could be more commensurate with theoretical estimates which suggest that the grains should be 400 million years older than the Solar System⁷.

A search continues for SiC grains which do not conform to the normal relationship in $^{29}\text{Si}/^{28}\text{Si}$ and $^{30}\text{Si}/^{28}\text{Si}$. Two such populations have now been observed, which S. Amari (Washington University) and colleagues have christened grains x and grains y. The latter population is only subtly different in isotopic composition from normal grains and their interpretation is not clear.

Grains x, on the other hand, show large enrichments in ^{12}C , ^{15}N , ^{26}Al and ^{28}Si . There are also excesses in ^{44}Ca and ^{49}Ti , thought to be the decay products of ^{44}Ti and ^{49}V respectively, which suggests they originated in a supernova. However, the apparent enrichment in ^{26}Al is not consistent with this picture; clearly the origin of these grains is not yet fully understood.

Thus the question remains: how many sources for the SiC? On the basis of the evidence we have thus far, it looks like only a limited number of discrete sites are necessary to explain the data. Undoubtedly as more SiC grains are analysed the story will become more complex and, indeed, more fascinating.

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A drug in a bug

DRUG-addiction creates an enormous amount of crime, misery, and social disruption. All of it could be avoided if addictive drugs were freely available. Once legalized, however, they would be rapidly spread by advertising and commercial enterprise, giving rise to new social problems. Daedalus now resolves the dilemma. He proposes a drug that you don't have to buy, and cannot sell.

His scheme exploits that miserable medical phenomenon, the permanent abscess or 'septic focus'. Before antibiotics, many people went for years with such chronic bacterial infections, typically in the inner ear or beneath a tooth root. So DREADCO biologists are creating a novel drug-bacterium. They are extracting the genes for cocaine synthesis from the coca-plant and genetically engineering them into *Staphylococcus*. An addict who is sick of the endless struggle to obtain an illegal drug, can simply apply to be stably infected with this drug-synthesizing organism.

Once the benevolent stable infection is leaking cocaine steadily into his bloodstream, the addict's troubles will be over. He will no longer be tempted into crime, or indeed any other activity. With his pleasure centres permanently satiated by internally generated drug, he will be absolved from the goal-seeking, trouble-avoiding pressures that beset the rest of us. He will decline the increasingly desperate offers of his distraught pusher with a pitying smile.

Intrinsically content, immune to threats, pleas and demands of any kind, 'auto-addicts' will be the most unworldly, even saintly, beings imaginable. They will still be a drain on the welfare services, but not seriously, and probably not for long. Their indifference to food and basic hygiene will steadily undermine their vitality. At any stage, of course, an auto-addict could seek medication with antibiotics, so as to return to the worried, driven life of the responsible citizen — but few will bother. Meanwhile, the illegal drug business will simply collapse. Its heavy social burden of crime, propagation of AIDS and destruction of community, will be lifted.

At first Daedalus feared that his drug-bugs might spread disruptively into respectable society at large. But he now points out that their useless burden of cocaine synthesis will handicap them strongly. They will be unable to compete with more efficient, naturally occurring bacteria. The only way you could get infected would be by the deliberate effort of having a strong culture injected beneath a tooth root, or other suitable site. It would be the last deliberate effort you would ever make.

David Jones

Halogen lamp carcinogenicity

SIR — The risk of indoor pollution by chemicals and radionuclides is much in the news. Less attention is paid to hazards associated with exposure to harmful ultraviolet radiations emitted by certain illumination systems, which are used more and more extensively in the workplace and at home. The potent genotoxicity of the light emitted by uncovered quartz halogen lamps was demonstrated recently both by using a mutagenicity test in *his⁻ Salmonella typhimurium*¹ and a DNA repair test in *Escherichia coli*.² At variance with solar irradiation, exerting mutagenic effects over a broad ultraviolet range, and with fluorescent light, delivering mainly ultraviolet B emissions, the mutagenicity of halogen lamps is due to far-ultraviolet wavelengths¹. Moreover, the spectrum of sensitivity of *E. coli* strains lacking DNA repair mechanisms is similar for halogen lamps and a monochromatic ultraviolet (254 nm) source². We observed some residual mutagenicity even after filtering both natural and artificial light through various cloths, but mutagenicity was prevented by glass and plastic covers.

These findings prompted us to investigate the carcinogenicity of halogen lamps using animal models. We recently started a large study, using MF1 and SKH-1 hairless mice, after completing a pilot study with SKH-1 adult mice in which four control mice were kept under natural light-dark cycle. Four mice were exposed 12 h per day at 50 cm distance from a 12 V, 50 W uncovered halogen quartz lamp equipped with a dichroic mirror, accounting for an illuminance of 10,000 lux. Under these conditions a mutagenic response was induced in *S. typhimurium* in less than 1 min. A further group of four mice was exposed to the same lamp, but a 2-mm-thick transparent glass sheet, totally preventing genotoxic effects, was interposed close to the lamp. After 12 months, the appearance of these mice and of the four controls was normal. By contrast, all four animals exposed to the uncovered lamp started to develop skin lesions, the earliest appearing after 3–4 months and growing in size and number, up to 15–20 per animal, until overlapping and covering large portions of the regions of skin directly exposed to the light. Skin tumours of up to 3 cm in diameter were detected. Histological analysis revealed the presence in all four animals of relatively benign forms, such as papillomas and appendage/basal tumours, as well as atypical squamous cell proliferation of increasing malignancy — keratoacanthoma-like tumours, carcinomas *in situ* and squamocellular carcinomas (P. Fial-

lo, personal communication).

Although based on only 12 animals, the results obtained in our pilot study are striking and fully confirm the expectations of *in vitro* genotoxicity studies. Extrapolation of animal data to humans is not straightforward. Nevertheless, the illumination doses and times in our study, although high, are not so far from the levels to which some people are exposed, particularly in the workplace.

Note that the aforementioned genotoxicity data, as well as physical measurements¹, provide evidence for the emission by halogen lamps of far-ultraviolet radiation, which is likely to be the range of wavelength inducing melanoma³. It is also important that the light emitted by halogen lamps is much more genotoxic than sunlight^{1,2}, which is

well known to contribute to cancer prevalence and mortality in humans⁴, and than fluorescent light, whose carcinogenicity is under debate⁵. Luckily, prevention is simple. Suitable glass or plastic covers, already used in some commercially available models, should be made compulsory for all new halogen lamps, and should be installed on all lamps already in use.

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Li in V404 Cygni

SIR — Casares *et al.* report¹ that the X-ray transient GS2023+338, which has been identified with V404 Cygni, must have a mass of at least 6.26 ± 0.31 solar masses and hence is likely to be a black hole. They report that the spectrum of the companion, from whose orbit they derive minimum masses, may be either that of a G9 (± 1)V dwarf star or of a K0 (± 4)III giant star. The nature of the companion may be used to establish the distance to the system and hence the X-ray luminosity during the outburst. Figure 2 of ref. 1 indicates that the companion may show the Li I resonance line at 6,707.8 Å. If that identification is correct, the cool star is most likely to be a young dwarf such as that found in the Pleiades^{2,3}, rather than a K giant. The V404 Cyg system is then 10× closer than the model favoured in ref. 1, relieving

the problem of a fantastically large X-ray luminosity at outburst.

To establish the wavelength of the possible lithium line, we have measured all the prominent dips in the spectrum of V404 Cyg as displayed by Casares *et al.*¹ with a transparent ruler. The wavelengths, solar wavelengths⁴ and displacements are shown in the table. For blended lines, the solar wavelength is a mean weighted by the equivalent width. The mean of all $\Delta\lambda$ s of identifiable lines is 1.91 ± 0.48 Å (mean error, m.e.), and the mean $\Delta\lambda$ of the seven lines between 6,630 and 6,730 Å (hence closest to the Li I line) is 1.85 ± 0.37 Å (m.e.). Using these values to correct the measured wavelength of 6,706.1 Å yields 6,708.01 or 6,707.95 Å, which are within 1 m.e. of the intrinsic wavelength of the Li I blend, 6,707.83 (assuming no ⁸Li to be present and the ⁷Li lines to be saturated). A similar set of measurements of

WAVELENGTH MEASUREMENTS OF V404 CYGNI

λ_{V404}	$\lambda_{\odot}$	$\Delta\lambda$	λ_{V404}	$\lambda_{\odot}$	$\Delta\lambda$
6,352.7	6,355.03	2.33	6,506.8	?	—
63.6	b1	—	14.1	?	—
68.1	?	—	16.8	18.37	1.57
90.7	93.61	2.91	90.7	92.93	2.23
97.5	6,400.00	2.50	95.6	97.87	2.27
6,406.3	08.03	2.00	6,606.8	6,609.12	2.32
10.0	11.66	1.66	10.9	?	—
20.0	20.75	0.75	15.4	?	—
29.0	30.86	1.86	22.7	?	—
37.9	39.08	1.18	32.0	33.76	1.76
48.4	49.82	1.42	42.0	43.64	1.64
53.4	55.60	2.20	51.2	?	—
60.7	62.60	1.90	61.8	63.40	1.60
69.5	71.67	2.17	76.3	78.00	1.70
74.3	75.63	1.33	6,701.8	6,703.58	1.78
80.0	82.18	2.18	06.1	—	—
92.9	94.49	1.59	15.9	17.69	1.79
6,497.7	b1	—	24.0	26.67	2.67
			6,736.8	?	—

Values from Fig. 2 of ref. 1. Numbers in Å. $\lambda_{\odot}$ is the solar wavelength.

the displayed spectrum¹ of the G9V star HR1232 yields a wavelength of 6,704.69 $\pm$ 0.28 for the feature nearest the lithium line, which is probably a blend of the 6,703.58 and 6,705.11 Å lines of Fe I whose mean, when weighted by the equivalent widths in the solar atlas⁴, is 6,704.23.

The lithium in V404 Cyg may be related to the fact that it is a binary with a period not too different from those of the RS CVn stars, which often show strong lithium lines despite their spectral types being about K0IV (ref. 5). But for RS CVn stars with $T_{\text{eff}} < 5,000$ K, the lithium abundance as given in Fig. 11 of ref. 5 is $\log N_{\text{Li}} \leq 1.8$ for 42 of 45 observed stars. For stars whose Li I line shows the same strength as 6,719 of Ca I, $\log N_{\text{Li}} \approx 2.5$, hence it is much more likely that V404 Cyg is a main-sequence star and neither a giant nor a subgiant. In addition, RS CVn stars are binaries consisting of a K giant or subgiant and a main-sequence star, not a compact star.

If our identification of the line at 6,708.0 Å as Li I is correct, a comparison with neighbouring lines indicates that the companion must have a lithium abundance near that of Pleiades stars of similar spectral type ($\log N_{\text{Li}} =$ about 2.5 on the standard scale of $\log N_{\text{H}} = 12$)². This

indicates that the age of the V404 Cyg system is of the order of 10^8 years because the lithium abundance is sufficiently uncertain that lithium might not have been depleted from the interstellar value at all.

Our confirmation that V404 Cyg is a dwarf adds weight to the model that places V404 Cyg at about 1.5 kpc from the Sun and an outburst X-ray luminosity near 3×10^{38} erg s⁻¹. This limits the possible models of the system. As discussed in ref. 1, the period of 6.5 days leads to an orbit which is too large for mass transfer to be taking place through the inner lagrangian point. Hence the model of V404 Cyg as a triple system with an unseen companion orbiting a black hole with a period near 6 hours becomes more likely.

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Bacteria and eukaryotes

SIR — The suggestions that eukaryotes should be renamed synkaryotes¹ or eukarya² should be strongly resisted. The purpose of biological nomenclature is not to describe taxa but to give clear, consistent and unambiguous labels to them: stability is highly desirable, and nomenclatural codes have always sought to discourage unnecessary changes. Neither of these changes has any merit whatever. Forterre¹ is mistaken in supposing that 'eukaryote' implies that eukaryotes are derived from prokaryotes. Eukaryote simply refers to their 'well-developed nucleus', an entirely correct description. But even if it were not, that would be no reason to change their name. Biological nomenclature is replete with descriptively inappropriate and misleading names of taxa that are retained because to change them would be confusing and impair information retrieval and effective communication. If I met a woman named Violet Green I would not ask her to change her name if she were neither colour.

For this reason I am opposed to the tendentious and illogical change of Archaeobacteria to Archaea². Archaeobacteria has been so long and widely accepted and understood that it seems undesirable to change it. The proposed change follows the more widespread rec-

ognition that Archaeobacteria are less old and much less distinct from eubacteria than Woese and Fox³ originally thought. One might have supposed that if this should prompt any change, it would be to Neobacteria, or better still to Metabacteria, as suggested long ago⁴ by those who never accepted the dogma that this group was as old as eubacteria. The proposed name of Archaea, and the even more confusing renaming of Eubacteria simply as Bacteria, seem designed to promote the oft-repeated but unjustifiable view that archaeobacteria are not bacteria at all but represent, as originally claimed, 'a new form of life'. Woese has mistakenly and repeatedly asserted that his recognition and firm establishment of the kingdom Archaeobacteria (certainly a great and important breakthrough) invalidates the classical distinction between prokaryotes and eukaryotes. But as archaeobacteria fall within the scope of prokaryotes and bacteria by every criterion as classically defined⁵, it does nothing of the kind.

If Forterre really does not like the term prokaryotes, because 'pro' does indeed have the connotation 'before', why does he not, instead of proposing a totally unnecessary new name, simply call them Bacteria, as they have been known for more than 100 years? The renaming of blue-green algae (a vernacular not a systematic name) as cyanobacteria made 'prokaryotes' simply a junior synonym for bacteria. More than 50

years ago, Prévot⁶ wrote, "*Pourquoi ne pas avoir le courage de dire: le Règne bactérien?*" I have therefore long adopted Bacteria as the formal scientific name for the superkingdom⁷, or empire^{8,9}, that includes the kingdoms Archaeobacteria and Eubacteria.

On the more substantive issue of whether bacteria are more primitive than eukaryotes, Forterre ignores the fossil evidence that they indeed are⁸. If he wishes his speculation that bacteria evolved by reductive evolution of eukaryotes (he actually says 'proto-eukaryotes', but I here take this vague term to mean 'first eukaryote') to be taken seriously, he should attempt to explain how they could have lost the cytoskeleton, endomembrane system and nuclear pores, as well as abolished mitosis and fused together their chromosomes into a circle and attached them to the plasma membrane, and drastically changed their DNA replication and segregation system. I have discussed⁹ the nature of the changes from the simpler bacterial cell to the vastly more complex eukaryotic one. Anyone who wishes to argue for the reverse ought to develop their hypothesis in enough detail for it to be shown to be mechanistically plausible (which I strongly doubt) and explain how it can be reconciled with palaeontological evidence^{8,9}.

The changes involved in forming a eukaryote from a bacterium are far more numerous and radical⁹ than those relatively much more trivial ones that separate eubacteria and archaeobacteria. For this reason the distinctions between bacteria and eukaryotes remain by far the most fundamental in the living world (other than the distinction between true, cellular organisms and viruses), and this primary division into two multikingdom empires (Bacteria and Eukaryota)^{8,9} is greatly to be preferred to a division into three domains². The latter has all the demerits of a one-character (one-molecule!) classification and ignores the numerous fundamental positive characters — both in genomic and in cellular organization^{7,9} — shared by all bacteria.

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Roller-bearing tectonic evolution of the Juan Fernandez microplate

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A combined GLORIA sidescan and Hydrosweep multi-narrow-beam sonar survey shows striking evidence for fan-shaped spreading systems, bounding pseudofaults and compression ridges associated with 90° of clockwise rotation of the Juan Fernandez microplate in the past 4 Myr. A quantitative analysis of this evolution suggests that the microplate has rotated as a rigid 'roller bearing' for most of its history, driven at its edges by shear between the surrounding major plates. The remarkable similarity between the Juan Fernandez and Easter microplates, despite their different tectonic settings, suggests that this is a general model for microplate tectonics.

THE Juan Fernandez microplate lies at the triple junction of the Pacific, Nazca and Antarctic plates, an open ocean area uncomplicated by hotspots, aseismic ridges or adjacent continents (Figs 1 and 2). It is roughly circular, about 300 km in diameter, and is one of several microplates along the East Pacific Rise. Many microplates form at junctions where three major plates meet, and one of the aims of our survey was to study the evolutionary history of such a triple junction.

The existence of a small plate at the triple-junction intersection of the Pacific, Nazca and Antarctic plates was postulated first¹⁻⁴ from a quasi-circular distribution of earthquake epicentres and from focal mechanism solutions suggesting motions that were not parallel or perpendicular to any of the major plate boundaries. Two specific reconnaissance surveys⁵⁻⁷ revealed the structure of the divergent Juan Fernandez-Nazca plate boundary (the East Ridge), where crust has accreted for the past 4 Myr, and of the northern portion of the divergent Juan Fernandez-Pacific plate boundary (the West Ridge), which contains a large overlapping spreading centre. These data were used to calculate Euler poles for the microplate relative to the surrounding major plates^{5,7}. All of the Euler poles lie on or within a few hundred kilometres of the microplate, implying a large rotational component of microplate motion. The earlier data were not, however, adequate for an understanding of the complex kinematic history of the microplate's evolution, which involves large and abrupt changes in rotational motion and in microplate boundary characteristics. We therefore undertook this survey and report the initial results here.

Our survey technique was to run parallel lines oriented east-west and spaced 33 km apart across the microplate, its boundaries and the areas surrounding the microplate that might have been formed at the microplate's boundaries or deformed by microplate processes (Figs 1 and 2). This track orientation optimized magnetic lineation definition, and the track spacing

allowed GLORIA sidescan insonification of the entire survey area. Hydrosweep data provided bathymetric calibration of the middle 5-8 km of each 33-km-wide sidescan swath and were combined with the GLORIA data to form merged sidescan sonar images. This was achieved by calculating a shaded relief representation of the Hydrosweep swath in which the sea floor was displayed as though illuminated from a constant 35° elevation in directions perpendicular to the ship track⁸. The pseudo-sidescan image was then merged with the GLORIA sidescan and conveniently filled the usual data gap in the GLORIA beam pattern directly beneath the instrument.

Kinematic models of microplates

The modelling of microplate kinematics began with adaptations of the propagating rift model⁹ to microplates. Early studies^{10,11} relied on the principle that microplate evolution occurs mainly as rift propagation between plates whose Euler poles lie at great distances from each other. An alternative suggestion¹²⁻¹⁴ was that this evolution occurs as a combination of rift propagation and microplate rotation about Euler poles near the propagating rift tips. This was expanded to include cases where the microplate was rigid or sheared during its evolution¹⁵. These models^{12,15} are not predictive, in that the exact locations of the microplate's Euler poles are not specified, nor are the spreading-rate variations along the propagating rift boundaries. Rather, they can be used to reconstruct the kinematic history of the microplate once the above parameters are known. A quantified, predictive model¹⁶ was developed from a special case of the rigid-microplate rotation scheme¹⁵. It predicts that a shear couple exists between two major plates and acts tangentially along the microplate boundaries, causing the microplate to rotate like a roller bearing. The Euler poles between the microplate and the major plates forming the shear couple lie at the propagating rift tips. The microplate rotates relative to the major plates at a high angular rate determined solely from the distance between Euler poles and the rate of shear between the two major plates. The model in which the Euler poles lie at the propagating rift tips^{15,16} can now be tested because the microplate rotation should satisfy the simple angular-velocity equation, $\omega = v/r$, where v is the relative velocity of motion between the two major plates; r is the distance between the propagating rift tips or the microplate's diameter; and ω is the microplate rotation rate calculated from the measured intersection angles between conjugate pairs of magnetic lineations across a microplate spreading centre.

Our survey allows us to evaluate r and ω as functions of time and, by combining these with measurements of v from other studies, test this hypothesis. If the measurements satisfy the above angular-velocity equation, then this suggests that the microplate is rigid, that its rotation is driven by horizontally directed shear stress along its edges and that mechanical coupling is perfect (no measurable loss caused by deformation as the tangential velocity of the major plate is transferred to angular velocity of the microplate).

A more conventional way (Fig. 3) to describe plate motion is to fix one plate and consider the relative motion of the other plates. We suggest that microplate rotation is driven today by shear stress from the Nazca and Antarctic plates, which are shown fixed in Fig. 3a and b. The distance between the Euler poles at the rift tips (JF/NAZ and JF/ANT) is the present-day value of the microplate's diameter (r). Note the predicted compression at the northern (Fig. 3a) and southeastern (Fig. 3b) boundaries of the microplate in each reference frame.

Rotation structures

Most of the tectonic and magnetic lineation fabric of the microplate and the surrounding area is dominated by the rapid, clockwise rotation of the microplate over the past few million years, which is still continuing (Fig. 3). This clockwise rotation can be verified from the fan-shaped magnetic lineation pattern generated at the East Ridge. Because conjugate pairs of lineations in this fan were parallel to each other when they formed at the East Ridge, and all major-plate motions are essentially translational, the included angle between their present-day trends is a measure of the total rotation of the microplate since the pair formed. For example, the triplet of lineations labelled 2A can be seen on either side of the East Ridge in Fig. 1. The outermost (oldest) lineation of the triplet formed at 3.4 Myr, and the included angle between this conjugate pair is about 80°. Thus, the total rotation of the microplate in the past 3.4 Myr is 80°, and the average rotation rate, ω , is 24° Myr⁻¹. Further examination of the East Ridge's magnetic lineation pattern shows that the actual rate of rotation of the microplate has varied, slowing down considerably between lineations 2 and J (for Jaramillo), between 1.66 and 0.98 Myr (refs 7, 17). The trends of the fan-shaped magnetic lineation pattern about the East Ridge are confirmed by the corresponding trends of the partially sedimented abyssal hills seen in the sidescan sonar mosaic (Fig. 2). It is clear that most of the acoustic relief in that area is initial spreading fabric, both because of its parallelism with the magnetic lineations and because of the gradual increase in sediment cover with increasing age.

There is a corresponding but oppositely directed fan of magnetic and abyssal hill lineations about the West Ridge, but both of these patterns are more diffuse than the ones about the East Ridge. Although magnetic lineations can be mapped and usually identified on the Pacific plate west of the West Ridge, nothing older than anomaly J has been recognized confidently on the microplate side. This seems to be due to a fracture zone that intrudes from beyond the western edge of the survey area, and to a discontinuous history of ridge jumping towards the east or to rift propagation events. Although we confirm previous conclusions⁵⁻⁷ that present-day spreading rates are considerably faster on the West Ridge than on the East Ridge, the opposite was true at the time of anomaly 2A, although the rates were more closely equal than at present.

These fan patterns of magnetic and abyssal hill lineations are bounded, or enclosed, by unconformities usually referred to as pseudofaults¹¹ which here mark the beginning of fan-shaped spreading in the microplate systems. Because the spreading systems tend to elongate by propagation at their rift tips, the lineations abutting the pseudofaults chronicle the history of that propagation and also the growth of the microplate diameter. The pseudofaults of the East Ridge (Fig. 1) occur where smooth basement relief abuts rough basement relief⁵, and the sidescan mosaic (Fig. 2) also shows a considerable change in trend of the abyssal hill fabric and in sediment cover.

The enclosing pseudofaults about the West Ridge lineation pattern are less obvious than their counterparts on the East Ridge. The West Ridge outer pseudofault (Fig. 1) trends north-west across the lineation pattern as a subtle ridge. The West Ridge inner pseudofault, although obscured by more recent volcanism, traces an inverted image of the East Ridge inner pseudofault. Enclosing the core of the microplate, the inner

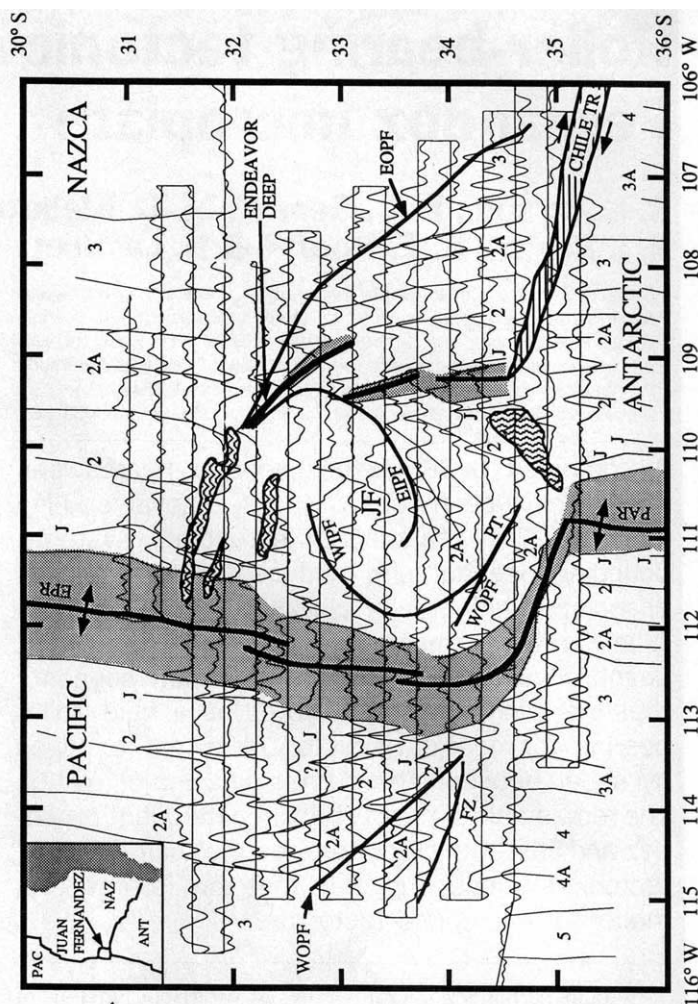
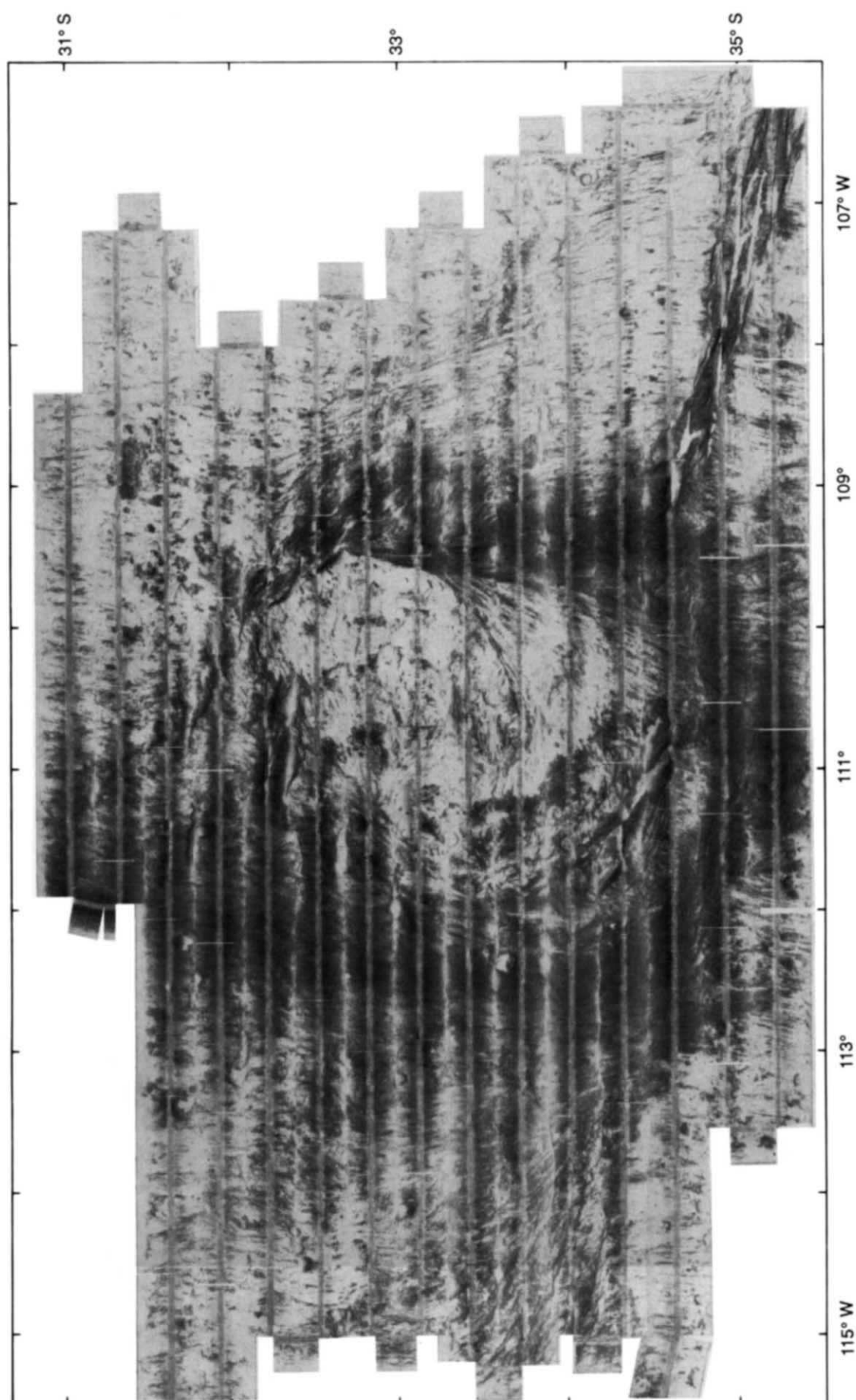


FIG. 1 Magnetic anomaly data collected during this study, with magnetic lineation correlations marked by fine lines. Some of these lines extend beyond the survey area on the basis of other data not shown here. Heavy lines show active plate boundaries, with the boundaries of the major plates labelled EPR for the East Pacific Rise (Nazca-Pacific), PAR for the Pacific-Antarctic Ridge (Pacific-Antarctic) and CHILE TR for the Chile Transform (Nazca-Antarctic). This last boundary is actually a zone ~25 km wide, indicated by hatching. The East Ridge and West Ridge are shown schematically in Fig. 3. Medium lines mark pseudofaults: EOPF, EIPF, WOPF and WIPF are the East Ridge outer, East Ridge inner, West Ridge outer and West Ridge inner pseudofaults, respectively; PT and FZ indicate a palaeo-transform fault and a fracture zone, respectively. The stippled region indicates crust created in the past 0.7 Myr. Three elongate compression ridges at the northern microplate boundary and the compressional/shear zone that forms the Juan Fernandez-Antarctic boundary are marked by wavy shading.

FIG. 2 Sidescan sonar mosaic of the Juan Fernandez microplate based on merged GLORIA sidescan and Hydrosweep multi-narrow-beam sonar data. Darker areas indicate areas of higher acoustic backscatter, interpreted as having higher proportions of bare volcanic rock or rough talus. Lighter areas indicate more sediment cover with sediments thicker than several metres, except in areas of steep relief where they may indicate shadow zones from slopes facing away from the ship track.



pseudofaults form a pattern resembling the meteorological symbol for a hurricane.

The history of propagation of the East Ridge is marked by the ages of the magnetic lineations that abut the inside of each pseudofault, beginning with anomaly 3 on the Nazca plate just north of the Chile Transform and extending to the present-day rift tips. Both the East and West Ridges terminate at present in rift-tip deeps with the East Ridge termination (Endeavor Deep¹⁸) being the deeper of the two. Endeavor Deep lies at the propagating end of a 150-km-long rift valley which trends northwest and bounds the northeast side of the microplate. This rift valley formed mainly by lithospheric extension and is flanked by elevated rims on both sides. Modelling of our gravity data is necessary for a more complete understanding. The two rift-tip terminations and many other features of the Juan Fernandez system resemble features on the Easter microplate^{19–21}; Endeavor Deep resembles the morphology and tectonic character of Pito Deep^{22–24}.

The eastern and western boundaries of the microplate are generally well delineated by spreading centres and propagating rifts, often with large, overlapping offsets. Unlike the Easter microplate to the north^{19,20}, no pure transform faults bound the currently active Juan Fernandez microplate²¹. The northern boundary is diffuse and has been predicted to be in compression⁵ like the Easter microplate^{12,15,19,20,25}. Three bulbous ridges, elongated east-west, are present in the sidescan data (Figs 1 and 2). They are up to 1 km high and have flanking deeps possibly indicative of flexed or folded crust and recent isostatic compensation. We interpret these ridges, previously attributed to a fracture zone⁶, as evidence for crustal shortening caused by compression²¹. These compressional features all lie completely on crust formed recently during the history of microplate rotation where compressional folding or buckling would have occurred with relative ease. The longest of these compression ridges also terminates just west of the Endeavor Deep rift tip. If this feature is a product of the present-day stress field, then the current Euler pole of the Juan Fernandez–Nazca plates is

somewhere between the tips of these compressional and tensional features (Figs 1 and 3a). Assuming rigid rotation, this important conclusion also partially predicts the current locations of the Euler poles for the Juan Fernandez–Antarctic and Juan Fernandez–Pacific plate motion¹⁶.

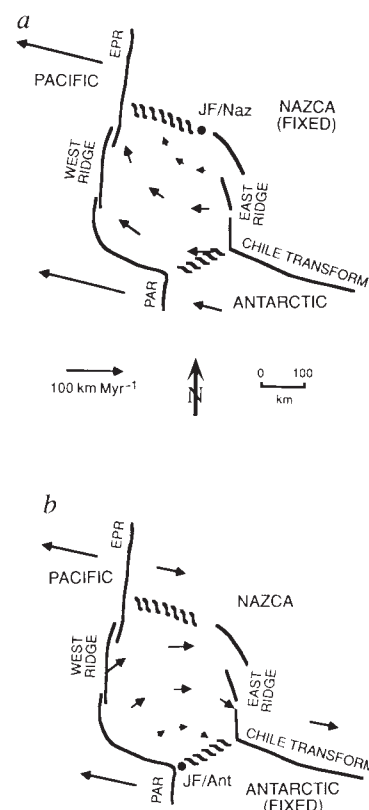
Microplate history

We suggest that the microplate's history began at the end of anomaly 3 as a large, overlapping spreading centre on the East Pacific Rise (Fig. 4a). The East Ridge extended from the overlapping spreading centre to the Chile Transform, as indicated by the anomaly 3 (3.88 Myr) lineation just north of the Chile Transform, which is truncated by the East Ridge outer pseudofault (Figs 1 and 4a). The West Ridge extended to the north as the East Pacific Rise as evidenced by the anomaly 3 lineation in the northwestern corner of our survey area (Fig. 1). This is the most poorly constrained part of our reconstruction because of our limited sampling of these magnetic lineations, and we cannot be certain of the original tectonic configuration. From our knowledge of the shapes and sizes of modern overlapping spreading centres^{26,27}, however, it seems logical to suggest²⁸ that microplates evolve from the largest of these structures.

At that time (Fig. 4a) the Pacific–Antarctic spreading centre (Pacific–Antarctic Ridge) was offset left-laterally along an extension of the Chile Transform to the Nazca–Pacific spreading centre (East Pacific Rise). The three major plates met at a ridge–fault–fault triple junction formed by the Pacific–Antarctic Ridge, the Chile Transform and its western extension. The present-day core of the microplate had just formed by growth of the overlapping spreading centre and was oriented $\sim 90^\circ$ counterclockwise to its present configuration (Fig. 4a).

By the onset of anomaly 2A at 3.40 Myr (Fig. 4b), the East and West Ridges had both grown by propagation as the microplate expanded by spreading from these boundaries. This dual propagation and expansion continued, accompanied by rapid rotation, until about the end of anomaly 2A (2.47 Myr, Fig. 4c)

FIG. 3 Arrows show the present-day relative plate motion of the Juan Fernandez microplate and two of the adjacent major plates when one plate of the shear couple that drives microplate rotation is fixed. Black dots locate relative motion poles and wavy lines show predicted zones of compression in each reference frame. *a*, Nazca plate fixed, showing relative motion (rotation) of the microplate about the JF/Naz Euler pole. *b*, Antarctic plate fixed, showing microplate rotation about the JF/Ant Euler pole.



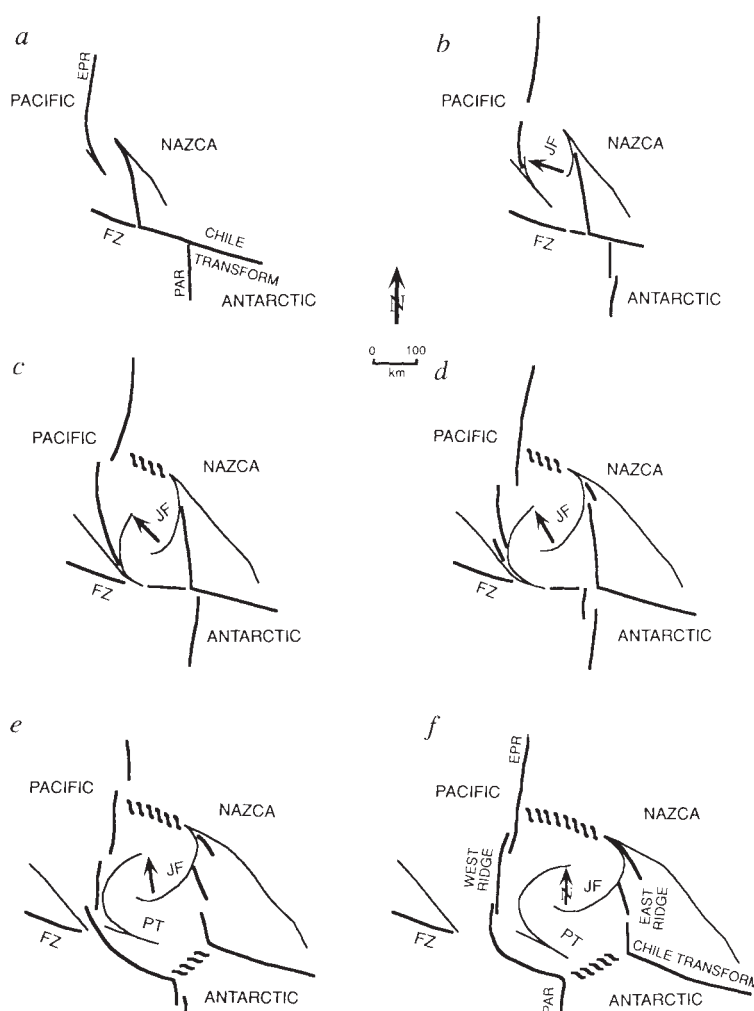


FIG. 4 Schematic tectonic reconstructions of the Juan Fernandez microplate area showing plate boundaries active at each reconstruction time and the associated pseudofault traces of rift propagation. *a*, Anomaly 3, 3.88 Myr; *b*, anomaly 2A (Old), 3.40 Myr; *c*, anomaly 2A (Young), 2.47 Myr; *d*, anomaly 2, 1.75 Myr; *e*, anomaly J, 0.95 Myr; *f*, present. The reconstructions restore conjugate magnetic lineations and pseudofaults to the congruent plate boundary locations at their times of origin. The Pacific plate was kept fixed in this exercise. FZ is the fracture zone extension of the Chile Transform, isolated by West Ridge propagation. The short arrow on the microplate points north at present. On earlier reconstructions it indicates the amount of microplate rotation still to occur between that reconstruction time and the present.

when the propagating tip of the West Ridge reached the fracture zone extending west from the Chile Transform. This halted the rapid growth of the microplate's diameter by rift-tip propagation, and marked the end of the first phase of the microplate's evolution (Fig. 5). During the second phase, the microplate evolved mainly by constant rotation at $\sim 32^\circ \text{Myr}^{-1}$. The rate of microplate rotation indicates that it was rotated clockwise by the right-lateral shear couple between the Nazca and Pacific plates at its north and south boundaries (Figs 4c and 5). This probably also initiated compression at the microplate's northern boundary.

The kinematics of the major plates require that the spreading centres migrate to shorten the intervening transform fault to the point of elimination. This was the case from anomaly 3 to anomaly 2. By anomaly 2 at 1.75 Myr (Fig. 4d), the Pacific–Antarctic Ridge had aligned with the East Ridge, eliminating the Nazca–Pacific part of the Chile Transform. Some time between anomalies 2 and J, the tip of the West Ridge was abandoned, and a new boundary developed ~ 50 km to the south, capturing a piece of Pacific plate of 2A age, which now lies at the southwestern corner of the microplate. This reorganization of the West Ridge marked the end of the second phase of microplate evolution (Fig. 5) and was followed by a change in the major-plate shear couple for microplate rotation from the Nazca–Pacific to Nazca–Antarctic plate pairs. The change probably resulted from the initiation and growth of the Juan Fernandez–Antarctic boundary. Microplate rotation slowed to $\sim 9^\circ \text{Myr}^{-1}$, as indicated by the geometry of magnetic lineations preserved about the East and West Ridges.

The microplate began its third (present) phase of evolution just after the time of anomaly 2 (Fig. 4d and 5). At about the time of anomaly J (0.95 Myr, Fig. 4e), the spreading direction changed on the East Ridge and the spreading rate slowed. To compensate for this, the spreading rate simultaneously increased substantially on the West Ridge. During the past 0.7 Myr, the East Ridge has continued to propagate to the northwest, forming Endeavor Deep, and the West Ridge has continued to create crust at its southern end. The formation of compression ridges continues by folding or buckling at the northern edge of the microplate.

It is difficult to predict the fate of the microplate because both spreading systems seem to be undergoing renewed activity in different ways. The spreading rate on the West Ridge has just increased markedly, and the East Ridge continues to propagate, forming Endeavor Deep (Fig. 4f). One of these spreading systems may eventually survive the other and raft the microplate away from the triple junction area on either the Nazca or Pacific plate. Another possibility is that both spreading systems will continue, and the Juan Fernandez–Antarctic boundary will be deactivated, welding the Juan Fernandez area to the Antarctic plate. Such a process probably caused the migration of the Pacific–Nazca–Antarctic triple junction from the Heezen fracture zone in the Cretaceous period²⁹ to its present location. The major-plate triple junction would then move to the northern edge of the Juan Fernandez area as a continuation of that northern migration. This might occur by similar microplate growth of the large, overlapping spreading centre now present at 29°S on the East Pacific Rise^{28,30}.

Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein

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PrP^C is a host protein anchored to the outer surface of neurons and to a lesser extent of lymphocytes and other cells. The transmissible agent (prion) responsible for scrapie is believed to be a modified form of PrP^C. Mice homozygous for disrupted PrP genes have been generated. Surprisingly, they develop and behave normally for at least seven months, and no immunological defects are apparent. It is now feasible to determine whether mice devoid of PrP^C can propagate prions and are susceptible to scrapie pathogenesis.

A NOVEL mechanism has been proposed to explain pathogenesis and transmission of spongiform encephalopathies such as scrapie in sheep and Creutzfeldt-Jakob disease or Gerstmann-Sträussler-Scheinker syndrome in man. The suggestion is that the infectious agent, or prion, is a modified form of PrP^C, a protein that is encoded by a single-copy host gene (*Prn-p*) and which is found on the outer surfaces of neurons and, to a lesser degree, of lymphocytes and other cells. The conversion of PrP^C into the modified form, PrP^{Sc}, is thought to come about either by interaction with exogenously introduced PrP^{Sc}, or, in the case of sporadic or familial disease, by a very rare spontaneous event which triggers a catalytic conversion cascade. The nature of the conversion is unknown, but it has been suggested that it might be conformational¹.

Several lines of evidence document the central role of PrP^C in scrapie-like diseases. The transmissible agent and PrP^{Sc} co-purify in different procedures²⁻⁶, including affinity purification on an anti-PrP antibody column⁷. Further, the susceptibility of a host to scrapie is determined, at least in part, by the nature of its *Prn-p* alleles⁸⁻¹³. Finally, certain mutations in the *Prn-p* gene, such as a proline-to-leucine codon change in position 102, are tightly linked to morbidity in some familial forms of spongiform encephalopathies^{3,14}, and mice carrying a *Prn-p* transgene with the analogous amino-acid change succumb to a spontaneous scrapie-like disease¹⁵.

The function of PrP^C is not known; the suggestion¹⁶ that it is identical to acetylcholine receptor-inducing activity remains to be confirmed. PrP^C seems to be physiologically important because (1) its gene has been found in all mammals examined (hamster¹⁷, mouse¹⁸, man^{19,20}, rat²¹, cattle²², sheep²¹ and goat²¹) as well as in the chicken (ref. 16, and J. M. Gabriel and S.B.P., unpublished results); (2) it is expressed in neurons of the brain²³ and other tissues²⁴, and (3) it has a high turnover rate^{25,26}. During embryogenesis PrP^C is expressed both in the central and in the peripheral nervous system and in specific non-neuronal populations, such as tooth bud, developing metanephric cap derivatives and placenta²⁷. Here we report that, contrary to expectation, disablement of both *Prn-p* alleles does not impair

normal development and behaviour in the mouse up to at least seven months of age.

Mice homozygous for disrupted *Prn-p* genes

We disrupted one *Prn-p* allele of murine embryonic stem (ES) cells by homologous recombination²⁸⁻³¹ with a 4.8-kilobase (kb) DNA fragment in which codons 4 to 187 of the 254-codon open reading frame were replaced by a neomycin phosphotransferase (*neo*) gene under the control of the herpes simplex virus thymidine kinase (HSV TK) promoter²⁹ (Fig. 1b). In the resulting construction, the first three *Prn-p* codons, the *neo* coding sequence and the residual 67 *Prn-p* codons were fused in-frame, with one nonsense codon interposed between the initial *Prn-p* codons and the *neo* sequence, and two nonsense codons between the latter and the residual *Prn-p* sequence (Fig. 1d).

The frequency of homologous recombination with this construct, as determined by polymerase chain reaction (PCR), was 1 in about 5,000 G418-resistant colonies. PCR (data not shown) and Southern analysis (Fig. 2a) of clone ESΔP-37/10 confirmed that one *Prn-p* allele was disrupted. Blastocysts of C57BL/6J (black) mice were injected with ESΔP-37/10 cells and implanted into foster mothers. About 45% of 52 offspring were partially, and 35% more than 90%, chimaeric with regard to agouti coat colour. Of 22 fertile males, 9 high-grade and 3 medium-grade (20-30%) chimaeras transmitted the agouti marker to their offspring. Screening of 166 agouti offspring by PCR analysis showed that about 50% were heterozygous for the disrupted *Prn-p* allele (data not shown). The presence of the disrupted gene (*Prn-p*⁰) was confirmed by Southern analysis (Fig. 2b). Only a single copy of the targeting sequence had been integrated, as shown by the fact that a *neo*-specific probe hybridized to only a single *Xba*I fragment of the expected length, 4 kb (Fig. 2b, bottom). Because the targeting sequence contained only a single *Xba*I site, each integration event should yield an *Xba*I fragment of different length, and tandem copies would yield additional fragments of 4.8 or 5.4 kb depending on their orientation relative to each other.

Prn-p^{0/+} heterozygotes were mated and 176 superficially indistinguishable offspring analysed by PCR (data not shown). To our surprise, 24% of these were homozygous for the disrupted *Prn-p* gene; 50% were heterozygous and 26% were wild type. The identification of homozygous *Prn-p*^{0/0} mice was confirmed by Southern analysis (Fig. 2c, lanes 3, 6 and 7). The average lifespan of these homozygotes has not yet been determined, but no spontaneous deaths were recorded up to 7 months.

Northern analysis of brain RNA from *Prn-p*^{0/0} homozygotes revealed a product of ~2.4 kb which hybridized with both a *neo* probe (Fig. 3b) and a *Prn-p* probe comprising the 3' part of the coding sequence still present in the disrupted gene (probe A, Fig. 3c), but not with a probe corresponding to the deleted gene segment (probe delta, Fig. 3a). This shows that substantial quantities of a fused messenger RNA containing the *neo* and the residual *Prn-p* sequence were present in the brain of *Prn-p*^{0/0}

homozygotes, and implies that no RNA cleavage and polyadenylation occurred at the *neo* polyadenylation site, or at least that no such mRNA accumulated. Brain RNA of heterozygotes contained both wild-type *Prn-p* mRNA and the *Prn-p-neo* fusion RNA at a lower level than in wild type and *Prn-p*^{0/0} homozygotes, respectively (Fig. 3c).

Western analysis of brain proteins showed that a set of bands present in wild-type samples was absent in *Prn-p*^{0/0} samples and present at about half the level in *Prn-p*^{0/+} samples (Fig. 3e).

Anatomy

No gross abnormalities, as judged by the size, weight and appearance of the brain, skeletal muscle and visceral organs, in particular of the spleen, were evident in the homozygous *Prn-p*^{0/0} mice, nor was their behaviour in any way unusual. The structure of the brain was normal, as judged by microscopic examination of haematoxylin-eosin-stained sections. About one third of the mice showed coarse vacuolization in the Ammons horn of the hippocampus, but the incidence of vacuoles was similar in wild-type, heterozygous or homozygous *Prn-p*^{0/0} animals (data not shown). Skeletal muscle was also normal, as judged by histochemical analysis of fibre types and distribution of sarcoplasmic reticulum (data not shown). Because skeletal muscle development and innervation appear normal in *Prn-p*^{0/0} mice, it is unlikely that PrP^C functions as a unique trophic factor targeted to muscle¹⁶.

Homozygous *Prn-p*^{0/0} mice are fertile and normal progeny resulted from homozygous *Prn-p*^{0/0} breeding pairs.

Immunological status

It has been reported that PrP^C is expressed on the surface of B and T lymphocytes and that it participates in lymphocyte activation, because an antiserum raised against PrP^C inhibits concanavalin A-stimulated lymphocyte proliferation²⁴. We therefore compared some immunological parameters of 6-week-old *Prn-p*^{+/+} and *Prn-p*^{0/0} mice. Thymocytes and splenocytes were analysed by cytofluorometry for expression of cell-surface IgM, CD3, CD4/8, as well as major histocompatibility complex (MHC) class I and class II antigens. Disruption of the *Prn-p* gene had no detectable effect on the level of these cell-surface markers (data not shown), indicating that PrP^C is not essential for the normal maturation of the lymphocyte subsets in either the thymus or the spleen. No significant difference in the response of splenocytes from *Prn-p*^{0/0} and *Prn-p*^{+/+} mice to activation by concanavalin A was detected (data not shown). This finding is in agreement with the report³² that glycosylphosphatidylinositol-anchored proteins, to which PrP belongs³³, are not required for activation of T cells by concanavalin A or antigens.

Behaviour

Because PrP^C is predominantly a neuronal protein and present in a high proportion of hippocampal nerve cells³⁴, the learning ability of *Prn-p*^{0/0}, *Prn-p*^{+/+} and *Prn-p*^{0/+} mice, all derived from the mating of the first generation of heterozygotes, was compared in three tests. In the swimming navigation, or swim test, the animal is placed in a pool and must learn to find a submerged

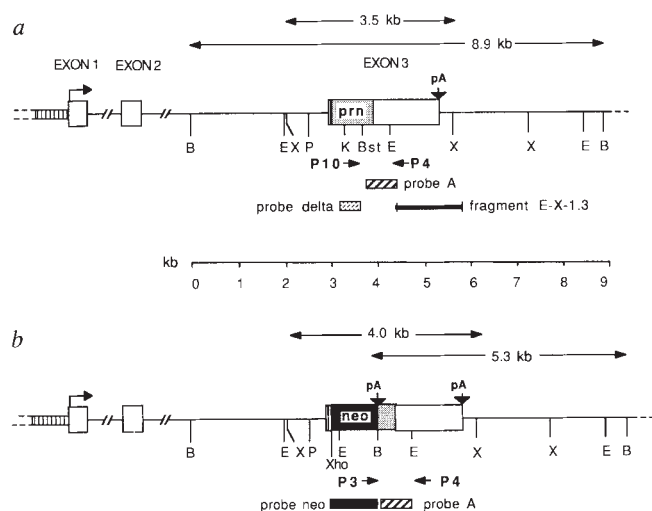
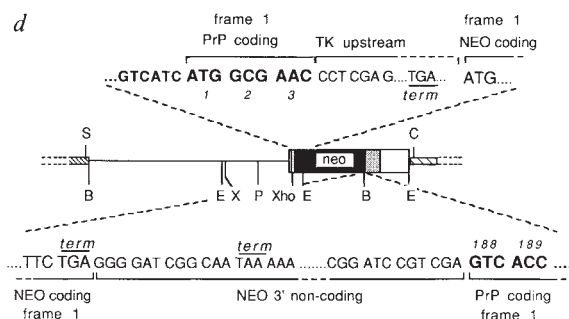
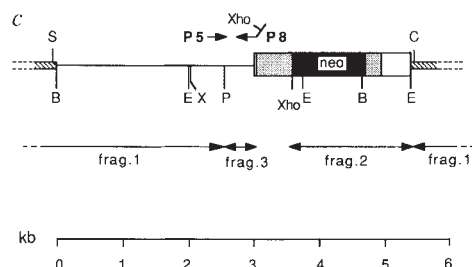


FIG. 1 Disruption of the *Prn-p* gene of murine ES cells. **a**, Map of the murine *Prn-p* gene (derived from refs 48 and 59). Not all *Pst*I (P) and *Bst*EII (Bst) sites are shown. **b**, Map of the disrupted *Prn-p* gene. The targeting vector pRV PrP-3 (**d**) was constructed by replacing 552 bp of the *Prn-p* coding region (extending from position 10 to 562, numbering relative to the first nucleotide of the initiator codon) by a 1.1-kb cassette containing the TK promoter followed by the *neo* gene. **c**, Construction intermediate; **a** and **b** show the primers used to identify the wild-type (P10 and P4) and the disrupted *Prn-p* allele (P3 and P4), respectively, as well as the probes for Southern analysis of wild-type and disrupted *Prn-p* genes (probes A, E-X-1.3) and northern analysis of *Prn-p*-related transcripts (probes A, delta and neo). B, *Bam*HI; Bst, *Bst*EII; C, *Cl*AI; E, *Eco*RI; K, *Kpn*I; P, *Pst*I; S, *Sac*II; X, *Xba*I; Xho, *Xho*I.

METHODS. pUC18 containing an 8.9-kb *Bam*HI fragment of mouse *Prn-p* genomic sequences including the PrP-encoding exon 3 (*Prn-p* clone NZW-*Bam*HI) was kindly provided by D. Westaway. The 4.2-kb *Bam*HI-*Eco*RI fragment was transferred to Bluescript to yield pBluescript-PrP2. The neoPA expression cassette from pMC1neoPA (Stratagene) was inserted into the blunt *Bst*EII site to give pRVPrNeo PA-2 (**c**). pBluescript-PrP2 was partially digested with *Pst*I, the full-length linear form was isolated and digested with *Cl*AI. The resulting 5.5-kb fragment 1 contained the Bluescript vector and *Prn-p* intron sequences between the *Bam*HI site and the *Pst*I site.



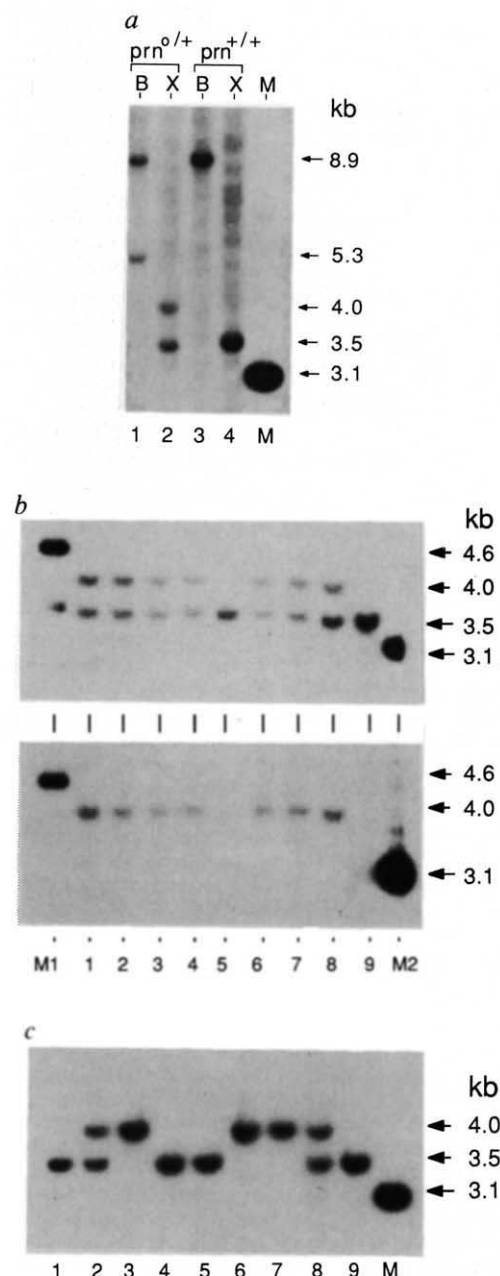
pRVPrNeo PA-2 was digested with *Xho*I and *Cl*AI, resulting in a 1.7-kb fragment 2 containing the neoPA expression cassette followed by *Prn-p* sequences between the obliterated *Bst*EII site in exon 3 and the *Eco*RI site in the 3' untranslated region and some base pairs of Bluescript linker up to the *Cl*AI site. Fragment 3, needed to reconstruct the region between the *Pst*I site in the intron and the *Xho*I site at the 5' end of the neoPA cassette, was prepared by PCR using linearized pBluescript-PrP2 as the template, the 5' terminal primer (P5) 5'-GCTTCTTCAAGTCTGTCTGCTGCTAG, which is complementary to intron sequences upstream of the *Pst*I site, and the 3' terminal primer (P8) 5'-TGACTCGAGGGTTCGGCATGATGACT, which is complementary to the intron-exon boundary and has an artificial *Xho*I site (underlined) at its 5' end (**c**). Digestion of the PCR product with *Pst*I and *Xho*I resulted in the 0.49-kb fragment 3, extending from the *Pst*I site to position +10 (relative to the PrP initiation codon). The 3 fragments were joined to give pRVPrNeo-3 (**d**). For transfection, the 4.8-kb disrupted *Prn-p* segment was excised with *Cl*AI and *Sac*II and purified.

platform with the aid of extramaze visual cues. Thereafter, the platform is moved and the ability to find the new platform location is assessed³⁵. Swimming navigation has high cognitive demands, is severely impaired after hippocampal lesions³⁶ and reveals behavioural correlates of genetically determined morphological³⁷ and physiological differences³⁸ in the intact hippocampus. There were no significant group differences in learning to find the submerged platform (Fig. 4a). After the location of the platform was changed, mice with *Prn-p*⁰ alleles, particularly the homozygotes, swam over the former location more frequently than their wild-type counterparts before finding the new position (Fig. 4a, legend). The Y-maze discrimination test as used here assessed the ability of mice to avoid punishment by means of a directional decision (go left or right) based on

visual and vibrissotactile cues presented randomly either in the left or right arm of the maze. It also measured the animal's ability to evaluate the efficiency of different problem-solving strategies³⁹. The overall performance of all tested animals was relatively poor, but there was a significant learning effect ($P < 0.001$). Wild types, hetero- and homozygotes showed no significant differences in discrimination learning (Fig. 4b). The two-way avoidance (shuttlebox) test is sensitive to both associative (learning-related) and non-associative factors (such as shock-sensitivity)⁴⁰. It is a sensitive indicator for hippocampal malfunction⁴¹. In this test all groups showed large behavioural variability, with a poor but overall significant learning performance ($P < 0.001$), which is typical for many mouse strains. The seemingly superior two-way avoidance learning

FIG. 2 Generation and characterization of ES cells and mice with disrupted *Prn-p* genes. **a**, ES cells (clone AB1, derived from agouti 129/Sv(ev) mice³¹) were transformed with the *Clal*-*SacII* targeting fragment shown in Fig. 1. Neomycin-resistant colonies were picked and placed into microtitre wells and the clone ESΔP-37/10 which contained a disrupted *Prn-p* gene was identified by PCR (data not shown) and confirmed by Southern analysis using probe E-X-1.3 (Fig. 1a). Lanes 1 and 2, ESΔP-37/10; lanes 3 and 4, untransformed ES cells; lanes 1 and 3, *Bam*HI-cleaved DNA; lanes 2 and 4, *Xba*I-cleaved DNA; lane M, marker (3.1 kb). **b**, Cells from clone ESΔP-37/10 were injected into blastocysts and chimaeric offspring, identified by agouti coat colour characteristic for the ES cell genotype, were mated to C57BL/6J females. Offspring heterozygous for the disrupted *Prn-p* gene were identified by PCR (data not shown) and confirmed by Southern analysis using *Xba*I-digested DNA. Lanes 1–4 and 6–8, *Prn-p*^{0/+} heterozygotes; lane 5, agouti wild type; lane 9, C57BL/6J (wild type); lanes M1 and M2, markers; top panel, probe A; bottom panel, probe neo. **c**, Heterozygotes were mated, the *Prn-p* genotype of the offspring was determined by PCR (data not shown) and confirmed by Southern analysis using *Xba*I-digested DNA and probe A (lanes 1, 4–5 and 9, wild type; lanes 2 and 8, *Prn-p*^{0/+} heterozygotes; lanes 3, 6 and 7, *Prn-p*^{0/0} homozygotes; lane M, 3.1-kb marker). The 4-kb band is diagnostic for the disrupted *Prn-p* gene and the 3.5-kb band for the intact gene (see Fig. 1a and b).

METHODS. AB1 ES cells were cultured on irradiated G418-resistant, LIF-expressing SNL76/7 feeder cells and passaged 1/6 every 2 days in DMEM with 20% FCS; both cell lines³¹ were provided by A. Bradley. About 3×10^7 trypsinized cells in 1.4 ml PBS in two cuvettes were electroporated with 10 μ g purified targeting fragment in each cuvette at 240 V, 500 μ F (Biorad Gene Pulser). Cells were plated onto ten 90-mm feeder plates and 0.3 mg G418 per ml was added after 24 h. After 10–12 days, G418-resistant colonies were picked and placed into individual wells of a 96-well plate, trypsinized and resuspended. Aliquots were plated onto irradiated feeder cells in 48-well master plates and the remaining cells of each well were pooled in groups of 12. About 50,000 cells were lysed³¹ and PCR was carried out as described⁶⁰ by cycling 35 times for 1 min at 94 °C and for 3 min at 70 °C, with primers P3 (ATTGCGACGCGATCGCTTCTATCGCC, complementary to the 3' end of the neoPA cassette) and P4 (CCTGGGAATGAA-CAAAGGTTTGCTTTCAAC, complementary to *Prn-p* genomic sequences adjacent to the targeting fragment) (see Fig. 1b). These primers give rise to a 850-bp product only when the targeting fragment is integrated correctly into the *Prn-p* locus. A positive control ES cell line ES-PC was generated by transforming D3 ES cells with a construct such as that shown in Fig. 1d, but containing additional 3' flanking sequences (the 1.3-kb *Eco*RI-*Xba*I segment shown in Fig. 1a), including the primer binding site for P4 which is absent from the targeting fragment. A pool of transformed ES cells containing a disrupted *Prn-p* gene was identified by PCR (data not shown) and the constituent clones were recovered from the master plate and analysed individually by PCR. The positive clone ESΔP-37/10 was expanded and analysed by genomic Southern blotting using probe E-X-1.3 (Fig. 1a). Chimaeric mice, identified by agouti coat colour, were generated essentially as described⁶¹ and mated at age 7 weeks to C57BL/6J females. Germ-line transmission was scored by agouti coat colour in the progeny. Mice were genotyped by PCR analysis of tail DNA⁶² using, in separate reactions, primers P3 and P4 to detect the disrupted *Prn-p* allele (850-bp band), and primers P10 and P4 to show the presence of the normal *Prn-p* allele (1.1-kb band). P10 (GTACCCATAATCAGTGGAAACAGCCAGC) hybridizes to the *Prn-p* sequences replaced by the neoPA cassette in the recombined allele. Samples were cycled 35 times for 1 min at 94 °C, for 2 min at 66 °C and for 1 min at 70 °C (data not shown). For Southern analysis, *Xba*I-cleaved DNA was blotted and hybridized⁶³ with either probe A (Fig. 1a,b) or probe neo, encompassing the neoPA sequence excised with *Xho*I and *Sal*I from pMC1neoPA²⁹.



of wild-type mice (Fig. 4c) was not statistically significant ($P = 0.32$).

The results of the behavioural tests show that $Prn-p^{0/0}$ and $Prn-p^{0/+}$ mice were not impaired in learning ability or in difficult tasks likely to reveal even minor brain damage. There were indications of a subtle difference in behaviour that needs to be confirmed and whose biological significance, if any, is a matter of interpretation: prolonged searching over a former target platform in the swimming navigation test may be taken as a measure of superior spatial memory or as a symptom of reduced behavioural flexibility. In any event, it is at present not possible to attribute any behavioural variation to the functional state of $Prn-p$ genes rather than to gene(s) linked to this locus. This is because the mice we have analysed are of mixed parentage and the $Prn-p^0$ allele is linked to loci derived from the 129/Sv(ev) mouse strain, whereas the $Prn-p^+$ allele is linked to loci of C57BL/6J mice.

Implications and outlook

The finding that mice devoid of functional $Prn-p$ genes show no abnormalities up to an age of at least seven months has significant implications. So far, it has been unclear whether the pathology of scrapie is due to the depletion of normal PrP^C or to the accumulation of PrP^{Sc} (refs 42–45) in infected neurons. The fact that mice can live normally for at least 7 months without expressing any PrP^C argues against the 'loss-of-function' hypothesis, although longer observation is necessary to confirm this. The possibility of raising mice devoid of PrP^C allows us to subject to a critical test the proposal that PrP^C is essential not only for the pathology associated with scrapie-like diseases, but

also for the formation of infectious agent. Moreover, the availability of $Prn-p^{0/0}$ mice provides a much improved approach to the analysis of foreign and mutant PrP genes^{15,46–48}. The introduction of exogenous $Prn-p$ genes into $Prn-p^{0/0}$ mice, either by mating with transgenic mice or by direct introduction of the gene, will allow the effect of different normal or mutant alleles to be studied without interference by the endogenous gene.

Should mice devoid of PrP^C indeed be resistant to scrapie infection, and if the expendability of these genes is a general phenomenon, it might be feasible to raise livestock impervious to the disease, a possibility of interest not only in animal husbandry, but also for the producers and consumers of cattle-derived products used in the preparation of many biopharmaceuticals. Moreover, if the pathology of spongiform encephalopathies is indeed due to the accumulation of PrP^{Sc}, therapy aimed at diminishing the synthesis of PrP^C would provide a rational approach to retard and mitigate disease progression, especially in familial forms where treatment could be instituted early.

Finally, it is interesting to speculate why a protein that is expressed in many areas of the brain and in other tissues, particularly during embryonic development, in all species of mammals examined and in at least one bird, and which shows a rapid turnover should be expendable without a detrimental effect. One other example of a conserved protein in a higher eukaryote whose ablation (by spontaneous mutation) does not lead to an abnormal phenotype is found in rats devoid of albumin⁴⁹. In lower eukaryotes, homozygous gene disruptions that fail to engender phenotypic changes are quite frequent; it has been estimated that in *Saccharomyces cerevisiae* almost half

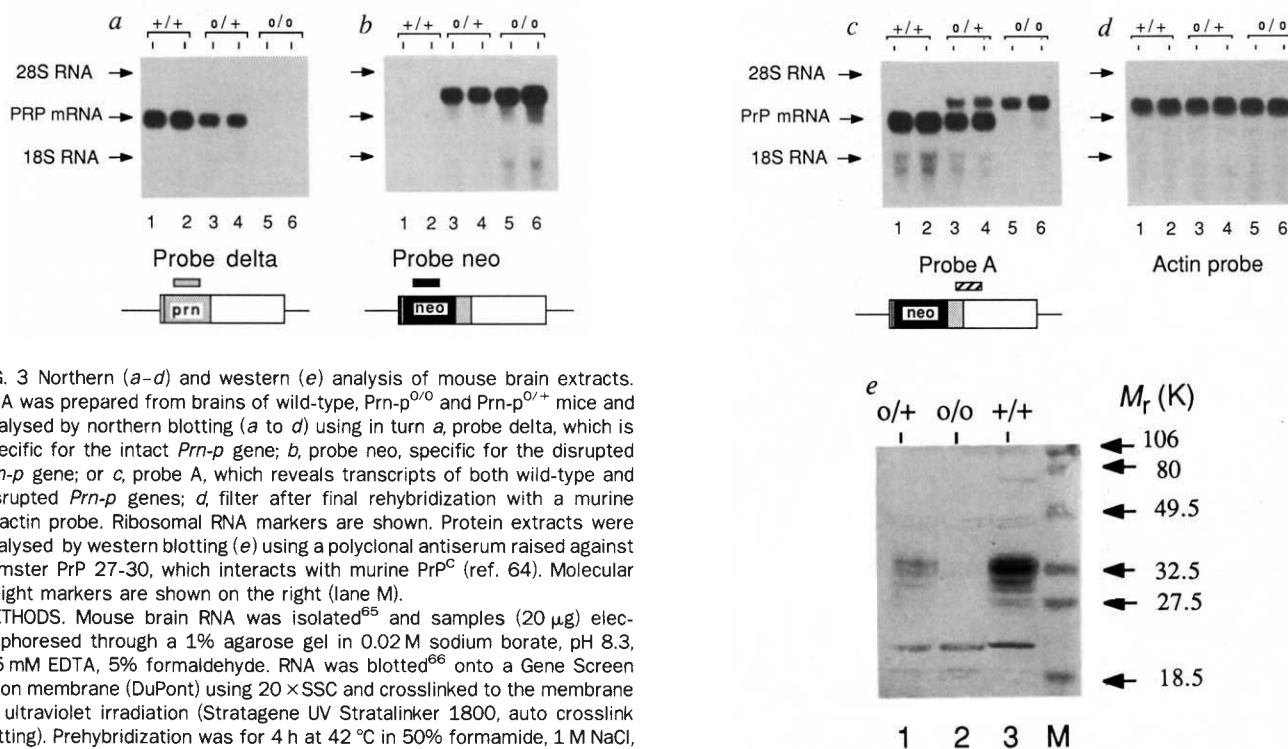


FIG. 3 Northern (a–d) and western (e) analysis of mouse brain extracts. RNA was prepared from brains of wild-type, $Prn-p^{0/0}$ and $Prn-p^{0/+}$ mice and analysed by northern blotting (a to d) using in turn a, probe delta, which is specific for the intact $Prn-p$ gene; b, probe neo, specific for the disrupted $Prn-p$ gene; or c, probe A, which reveals transcripts of both wild-type and disrupted $Prn-p$ genes; d, filter after final rehybridization with a murine β -actin probe. Ribosomal RNA markers are shown. Protein extracts were analysed by western blotting (e) using a polyclonal antiserum raised against hamster PrP 27–30, which interacts with murine PrP^C (ref. 64). Molecular weight markers are shown on the right (lane M).

METHODS. Mouse brain RNA was isolated⁶⁵ and samples (20 μ g) electrophoresed through a 1% agarose gel in 0.02 M sodium borate, pH 8.3, 0.5 mM EDTA, 5% formaldehyde. RNA was blotted⁶⁶ onto a Gene Screen nylon membrane (DuPont) using 20 $\times$ SSC and crosslinked to the membrane by ultraviolet irradiation (Stratagene UV Stratalinker 1800, auto crosslink setting). Prehybridization was for 4 h at 42 $^{\circ}$ C in 50% formamide, 1 M NaCl, 1% SDS, 10% dextran sulphate. Hybridization was overnight at 42 $^{\circ}$ C in the same buffer using ³²P-labelled probe (10⁶ c.p.m. per ml, prepared by random priming⁶⁶) and 0.1 mg ml^{−1} denatured salmon sperm DNA. The actin probe was a 250-bp *Pst*I fragment of mouse β -actin cDNA. The membrane was washed for 10 min at room temperature in 2 $\times$ SSC, twice for 30 min at 65 $^{\circ}$ C in 2 $\times$ SSC, 1% SDS, and twice for 30 min at room temperature in 1 $\times$ SSC. Autoradiography was for 7–38 h at −80 $^{\circ}$ C using Kodak XAR-5 film and an intensifier screen. For western analysis, samples (10 μ l) of a 10% (w/v) brain homogenate⁶⁴ were adjusted to 2% SDS and electrophoresed through a 1.5-mm 12.5% SDS-polyacrylamide gel (acrylamide:bisacrylamide, 1:20)⁶⁷. Proteins were transferred to nitrocellulose (Schleicher and Schüll, BA 85, 0.45- μ m pore size) by semi-dry electroblotting (IKA Biotech, Den-

mark). The membrane was preincubated for 2 h in TBSM (0.01 M Tris-HCl, pH 7.2, 0.15 M NaCl, 1% non-fat dry milk) (Carnation), incubated overnight at 4 $^{\circ}$ C in the same buffer containing a 1:5,000 dilution of the rabbit anti-hamster PrP 27–30 antiserum R073 (ref. 64), washed 3 times for 5 min in TBSM and incubated for 2 h in TBSM containing a 1:2,500 dilution of goat anti-rabbit IgG coupled to alkaline phosphatase (Promega). After washing in 0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, colour development was initiated in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 0.005 M MgCl₂ containing 0.33 mg ml^{−1} nitroblue tetrazolium (Promega) and 0.165 mg ml^{−1} 5-bromo-4-chloro-3-indolyl-phosphate (Promega), and terminated by washing in 0.01 M Tris-HCl, pH 8.0, 0.001 M EDTA.

of gene disruptions fail to display an obvious phenotype⁵⁰. Many examples have been described for *Caenorhabditis elegans*⁵¹ and some for *Drosophila*⁵². One explanation for the apparent expendability of a gene product is that the resulting defect is so subtle that a selective disadvantage may emerge only after many generations, perhaps under stressful natural conditions. Such functional defects might only become detectable by targeted assays, when one knows what to look for, as in the case of

HPRT⁻ (hypoxanthine phosphoribosyl transferase-deficient) mice⁵³, β_2 microglobulin-deficient⁵⁴⁻⁵⁶ or interleukin-2-deficient mice⁵⁷. Another possibility is that the function of the missing protein within the cell is assumed by related or different protein(s), or that the function is redundant. It is notable in *Drosophila* that protein null mutations of fasciclin I, a glycosylphosphatidylinositol-linked neuronal cell adhesion molecule, and of Abelson cytoplasmic tyrosine kinase do not

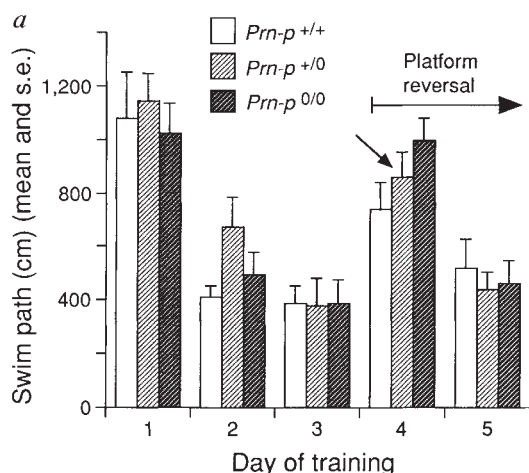
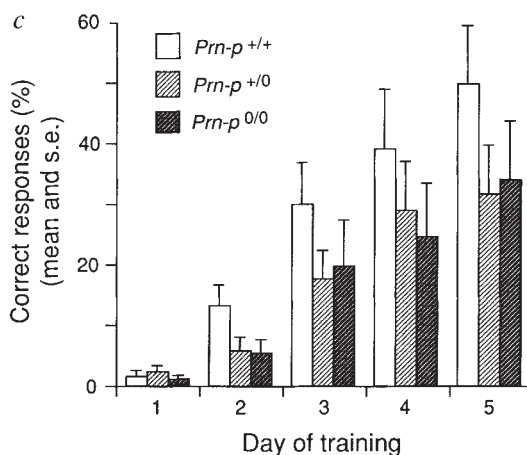
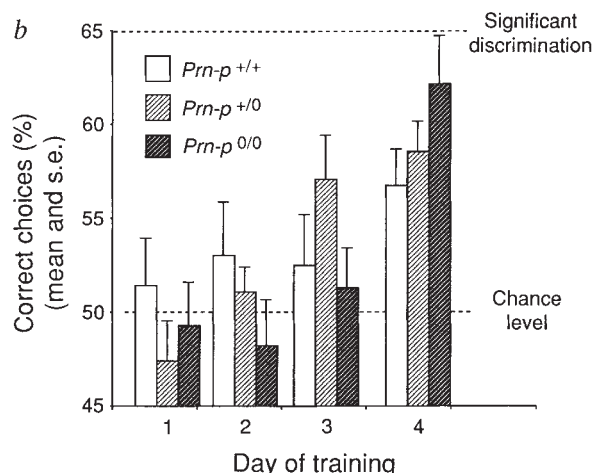


FIG. 4 Behavioural analysis of Prn-p^{+/+} wild type mice, Prn-p^{0/+} heterozygotes and Prn-p^{0/0} homozygotes in three learning devices. *a*, in the swim test³⁵, acquisition learning (days 1–3) and reorientation learning (days 4–5) by all mice was fast and did not reveal overall group differences. There were, however, large individual differences in every group. The average performance of the mice in this test corresponds to the one observed in the inbred strains DBA/2 and C57BL/6 (ref. 37). During the first day of platform reversal there was a significant allelic effect ($\rho = 0.35$, $P < 0.05$) for the average length of swimming paths (oblique arrows) in that Prn-p^{0/0} mice showed longer paths because of persistent crossings over the former position of the platform. *b*, in Y-maze discrimination learning, overall performance of the mice was variable and rather poor, indicating the inherent difficulty of the task which was mastered by only a few mice. Prn-p^{0/0} mice performed slightly better at the end of the training period ($\rho = 0.29$, $P = 0.08$). The chance level (no discrimination learning) is 50% for correct response, and significant discrimination beyond chance requires 65% correct responses in 36 trials. *c*, In two-way avoidance (shuttlebox learning), rodents must avoid an electric foot-shock announced by a warning light by running to the opposite chamber of a two-compartment box. The task imposes conflicting behavioural tendencies, and performance is strongly influenced by motor activity levels. The results reveal a slightly but nonsignificantly superior performance of Prn-p^{+/+} mice (group differences: d.f. 2, 33, $F = 1.16$, $P = 0.32$; repeated measures for learning effects: d.f. 2, 4, $F = 34.98$, $P = 0.0001$; no interaction effects). There was strong variability and no allelic ordering. Mice showing poor avoidance did not do so because of immobility responses ('freezing').

METHODS. All mice originated from 12 matings of first generation Prn-p^{0/+} heterozygotes, and were tested between 10 and 14 weeks of age. Twelve wild-type mice (6 males, 6 females), 13 heterozygotes (6 males, 7 females) and 11 homozygotes (6 males, 5 females) were coded and assigned randomly to three groups. Female mice were kept in groups of three and male mice were kept alone or with C57BL/6J females under a light cycle of 12 h (lights on from 08:00 to 20:00) and received food *ad libitum*. The testing of each group took three weeks. The order of tasks was swimming navigation, Y-maze learning, followed by shuttlebox conditioning. The test was in principle conducted under blind conditions, but 9 of 12 wild-type animals were recognizable by their black coat colour. Statistical comparisons were made by means of analysis of variance (ANOVA) with the training days as repeated measures to assess group and learning effects spanning the entire experiment. Because of the non-normal distributions of many behavioural scores, non-parametric ANOVA applied to the scores of a given day was used to verify the results of the parametric analysis. Allelic effects were checked by means of non-parametric correlations (Spearman's rho corrected for ties) between a dummy variable (0 for wild type, 1 for heterozygotes, 2 for homozygotes) and the behavioural scores⁶⁸. *a*, The swim test was done as described³⁷, except that a circular pool of 150-cm diameter was used and



all animals performed 6 trials a day. Mice were released from various locations in the pool (filled with water at 24–26 °C which was rendered opaque by addition of milk) and allowed to seek the submerged platform for at most 120 s. Between trials they were placed for 30–45 min under infrared lamps. Swim paths were tracked by a video camera and stored in a computer, the data being analysed off-line⁶⁹. The average swimming speed of the mice was about 25 cm per second. *b*, The computer-controlled Y-maze test is detailed elsewhere³⁹. Briefly, the mouse traverses a tubular maze in which it must choose between two arms whose sidewalls are formed by brass plates. The correct arm (discriminative stimulus) is signalled by brass plates with slits of 5 mm width, the wrong arm by smooth plates, which provides visual and vibrissotactile cues. The wall plates are swapped mechanically from trial to trial following a pseudorandom (Gellerman) sequence. Wrong choices are punished by air puffs (negative reinforcement). Formally this task can be classified as a discriminated avoidance learning procedure using non-spatial cue properties. Animals were allowed to adapt to the apparatus for 60 min on the first day of the experiment, and were then trained for discrimination learning on 4 subsequent days for 36 trials a day. *c*, Shuttle-box learning was performed in commercially available shuttle-boxes (Campden Instruments) placed in sound-proof enclosures and operated by a computer. The procedure was essentially as described⁴¹, except that the conditioning light stimulus lasted for 5 s and the inter-trial interval varied between 5 and 15 s. The animals performed 80 trials a day. The electric shock was 100 μ A (constant current measured in series with the animals), but required higher settings of the shock source because of polarity scrambling along the shock grids.

lead to gross defects, but that double mutants show a clear defect in growth cone guidance⁵². *En-2*-deficient mice, although they have a smaller cerebellum with an altered pattern of folding, show no functional abnormalities, perhaps because of functional

redundancy as a result of the structurally related *En-1* gene⁵⁸. It is even conceivable that a protein required earlier in evolution may no longer serve any function, and that its conservation is a result not of selective pressure but of evolutionary inertia.

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LETTERS TO NATURE

The origin of high-energy cosmic rays

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FERMI'S suggestion¹ that a population of low-energy charged particles could be accelerated by momentum exchange with 'wandering' galactic magnetic fields has long been a favoured mechanism for the generation of cosmic rays. A difficulty with this idea, however, recognized by Fermi² and others^{3–5}, is that unless the scattering centres of strong magnetic fields have velocities close to the speed of light, the acceleration mechanism is thought to be too slow to produce the highest-energy ($\sim 10^{20}$ eV) cosmic rays before particles escape from the Galaxy. We argue here that this problem is not intrinsic to the Fermi theory, but results only from approximating the essentially stochastic acceleration process as a deterministic one, in which particles diffuse upwards in energy at some mean rate. The stochastic treatment we present here suggests that the particles that acquire the highest energies are those on the wings of the statistical energy distribution, which proceed not in a pedestrian manner but are accelerated much more effectively as 'high flyers'.

The standard treatment of Fermi's idea as a second-order process considers all particles to diffuse gradually in energy according to

$$E_{\text{det}} = E_0 e^{N\beta^2} \quad (1)$$

where E_{det} is the energy attained after N scatterings, E_0 the initial energy and $\beta \ll 1$ is the mean velocity of scattering centres normalized to the speed of light, c . The exponent in equation (1) may also be written as ratio of elapsed time to an 'acceleration time'⁴ $\lambda/(c\beta^2)$, where λ is the mean free path between scatterings, assumed to be independent of energy.

Our analysis differs by considering the statistical range of possibilities for different particles. Fermi showed that each scattering would increase, or decrease, a particle's energy by a factor of $1 \pm \beta$. It is readily shown that a particle experiencing an excess of x energy-increasing scatterings in a total of N would acquire an energy of $E = E_0(1 + \beta)^x(1 - \beta)^{(N-x)/2}$. For $N\beta^2 \ll 2$ and $\beta \ll 1$, this approximates closely to

$$E = E_0 e^{x\beta} \quad (2)$$

Using the standard 'coin tossing' theory for the probability of an individual particle experiencing an excess of x positive scatterings in total number of N , and for the probability of experiencing N scatterings before escape from the scattering medium when the average for all particles is N_0 , we find that the probability of there being an excess of x is

$$P(x, N_0) = \frac{1}{N_0\sqrt{2\pi}} \int_{-\infty}^{\infty} \frac{1}{\sqrt{N}} e^{-x^2/2N} e^{-N/N_0} dN$$

Using equation (2) to write x in terms of E , and then integrating over N , we find that

$$P(E, N_0) = \frac{1}{\beta E_0\sqrt{2N_0}} \left(\frac{E}{E_0} \right)^{-(1+(1/\beta)\sqrt{2/N_0})}$$

TABLE 1 Effect of mean scattering-centre velocity

β	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
E_{lim}	2×10^{20}	5×10^{19}	10^{19}	2×10^{18}	eV
H_{crit}	6×10^{-2}	10^{-1}	3×10^{-1}	6×10^{-1}	gauss
λ	10^{21}	10^{20}	10^{19}	10^{18}	cm
a	10^{19}	10^{18}	10^{17}	10^{16}	cm
d	10^{20}	10^{19}	10^{18}	10^{17}	cm
T_S	5×10^{10}	5×10^9	5×10^8	5×10^7	s
N_0	50	5×10^3	5×10^5	5×10^7	
T_E	2×10^{12}	2×10^{13}	2×10^{14}	2×10^{15}	s
N_{10}	2×10^2	2×10^3	2×10^4	2×10^5	

Limiting energies, critical magnetic field strengths and other quantities (rounded values) are given for a range of scattering-centre velocities β normalized to the speed of light.

As $P(E, N_0) dE$ is identical to the equilibrium distribution that would obtain if particles were injected continuously, and this is in turn proportional to the differential energy spectrum, the above equation shows that the spectrum has the form of a power law with negative exponent

$$\gamma = 1 + \frac{1}{\beta} \sqrt{\frac{2T_S}{T_E}} \quad (3)$$

where, in accord with usual practice, N_0 has been replaced by its equivalent, the ratio of the average escape time, T_E , to the mean period between scatterings, T_S . This result satisfactorily reproduces the observed, roughly power-law form of the cosmic-ray spectrum, where $\gamma \approx 3$. We note, for comparison, that the exponent predicted by the deterministic approach is³

$$\gamma_{\text{det}} = 1 + \frac{T_S}{\beta^2 T_E} \quad (4)$$

and that equations (3) and (4), fortuitously, coincide in requiring $\beta^2 = T_S/(2T_E)$ to give $\gamma = 3$. We note also that, as the spectrum is of power-law form, a distributed spectrum of initial energies E_0 would lead to a composite spectrum of the same form at energies above the energies of injection. At lower energies, not under consideration here, the spectrum is likely to be dominated by the injection spectrum.

Acceleration can continue only as long as the fractional increase of energy due to a single scattering exceeds the radiation loss during scattering (radiation loss in the much weaker fields between scatterings is negligible). This constraint may be expressed, using the standard radiation time⁴ for protons of energy E in a magnetic field H , as

$$\beta \geq 8 \times 10^{-39} a E H^2 \quad (5)$$

where a is the radius (in centimetres) of a scattering centre, E is in electron volts and H is the scattering-centre field in gauss. It is also necessary for the field to be strong enough to deflect the particle significantly:

$$H \geq E/(300a) \quad (6)$$

The earlier (spectral-index) requirement may be expressed, for this random-walk process in which $T_E/T_S \approx (R/\lambda)^2$, where R is the typical linear dimension of the scattering medium, as

$$\lambda = \sqrt{2} \beta R \quad (7)$$

Combining equations (5)–(7), and putting $a = \delta \lambda$, where $\delta \ll 1$, we discover a limiting energy

$$E_{\text{lim}} \approx 2.5 \times 10^{14} (\delta R \beta^2)^{1/3} \quad (8)$$

and a critical magnetic field strength for the scattering centres

$$H_{\text{crit}} \approx 6 \times 10^{11} [\beta (\delta R)^2]^{-1/3}$$

For fields above H_{crit} , the limiting energy is given by equation (8), whereas for lower fields, the limiting energy will be reduced in proportion.

We can now explore these findings quantitatively in different situations. Table 1 shows E_{lim} and H_{crit} for $\delta = 10^{-2}$, $R = 10^{22}$ cm (the thickness of the galactic disc at the solar position) and $\gamma = 3$, for $10^{-1} \geq \beta \geq 10^{-4}$, together with corresponding values of other quantities appearing above. The typical spacing of the centres, $d \approx \lambda (\pi \delta^2)^{1/3}$, is also shown, as is the minimum number of scatterings to gain 10 decades of energy ($N_{10} = 23/\beta$). We note that, although for $\beta = 10^{-1}$, $N_{10} > N_0$, the probabilities of scattering and escape still ensure that, as for $\beta \leq 10^{-2}$, energization to the corresponding E_{lim} is prevented neither by escape nor by radiation losses. For $\beta = 10^{-4}$, T_E is equal to the collision lifetime in a medium of mean density one proton per cubic centimetre, $\sim 2 \times 10^{15}$ s; but this is not a limitation, as $N_{10} \ll N_0$. We conclude, therefore, that the Fermi theory, with its true stochastic nature taken into account, is fully capable in principle of accounting for proton acceleration to energies $\sim 10^{20}$ eV within the Galaxy (extragalactic cases have yet to be explored). In contrast to the energy gains of 10 orders of magnitude which Table 1 shows to be possible, the deterministic assessment suggests, as can readily be confirmed from equation (1), that for all cases shown in Table 1, energization would be by less than a factor of 2. To gain 10 orders of magnitude at the pedestrian rate the process would need to operate, again in all cases, for 40 escape times. The reason for this enormous disparity may be understood immediately on comparing the second-order general energization expressed in equation (1) with the first-order individual energization represented by equation (2).

In the light of our analysis, the well-established observation that the cosmic-ray spectrum extends as far as $\sim 10^{20}$ eV may be seen as *prima facie* evidence for the existence within the galactic disc of localized scattering centres with magnetic fields $\sim 10^{-1}$ gauss. $\square$

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Resonant orbital evolution in the putative planetary system of PSR1257+12

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PERIODIC variations in the arrival times of pulses from the millisecond pulsar PSR1257+12 are most straightforwardly interpreted as indicating the presence of two planet-like companions orbiting the pulsar¹. Rasio *et al.*² have proposed that the planetary explanation is amenable to a simple test: the deduced parameters put the planets near an orbital resonance, in which case secular evolution of the orbits should be observable in a matter of years. Detection of such orbital evolution would yield the masses and orbital inclinations of the planets. Here we point out that if the masses of the two planets are more than ~ 10 times greater than the minimum values (3.4 and 2.8 Earth masses) allowed by the observations, then their orbits will be in an exact 3:2 resonance. The character of the predicted orbital parameter perturbations is then markedly different from the periodic perturbations that result from only a near-resonance. The amplitude of the perturbations is much greater, and is very sensitive to the planet masses.

The masses of the objects orbiting the millisecond pulsar

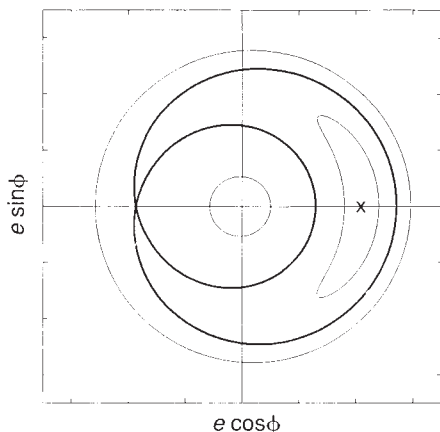


FIG. 1 Schematic of the structure of the phase-space trajectories near a first-order resonance. The distance from the origin measures the orbital eccentricity (e_1 or e_2), the polar angle measures the resonance phase (ϕ_1 or $\phi_2 - \pi$, respectively). The bold curve represents the resonance separatrix. If the planets are locked in resonance, the system trajectory librates about point X in the resonance zone.

PSR1257+12 are reported to be $2.8M_{\oplus}/\sin i$ and $3.4M_{\oplus}/\sin i$, where $M_{\oplus}$ is the mass of the Earth and i is the inclination of the orbital plane to the plane of the sky. To test the reality of these planets, the simplest consideration is that of the long-term stability of the system. To this end, we used an empirical stability criterion for general three-body systems³, and the resonance overlap criterion for the restricted three-body system⁴, to obtain the bound $1/\sin i \leq 180$. (These two stability criteria are identical for the case of the planar restricted three-body problem; R.M., unpublished work.) We have confirmed these estimates of the stability boundary by direct numerical integration. We used for initial conditions the set of orbital elements inferred from ref. 1, together with the assumption that the planets move in coplanar orbits. In addition, we assumed the canonical value of $1.4M_{\odot}$ for the mass of the pulsar. We integrated the orbits for 10^5 years using a fifteenth-order Gauss-Radau integrator⁵. Dynamical stability places an upper bound on the mass of each planet of twice the mass of Jupiter; a similar bound was obtained in ref. 2 by independent methods.

The orbital periods of the putative planets are reported to be 66.6 days and 98.2 days, close to a 3:2 ratio. Because of this near-commensurability of the orbital periods, the mutual perturbations of the planets may be large enough to be observable over a relatively short period of time. Rasio *et al.* argued that the orbital parameters should show periodic variations; the

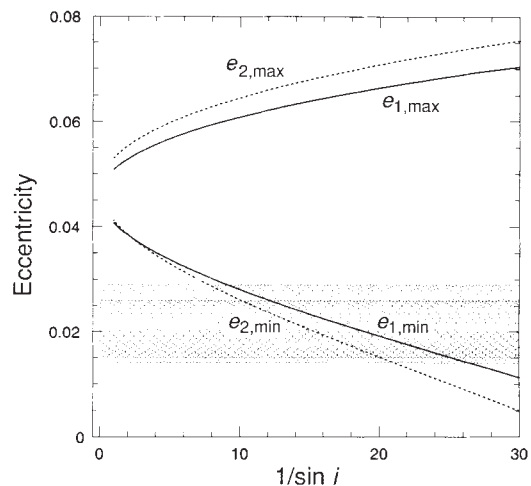


FIG. 2 The width of the resonance zone is characterized by the amplitude of the changes in the eccentricity on the separatrix. Shown are the minimum and maximum values of the eccentricity on the separatrix as a function of the mass factor $(\sin i)^{-1}$. The observed orbital eccentricities lie within the shaded horizontal bands.

amplitude of the secular and near-resonant perturbations is proportional to the ratio, m_p/M_s , of the mass of the perturbing planet to the mass of the central star, and hence is proportional to $1/\sin i$. But the maximum amplitude of the perturbations in exact resonance is proportional to $(m_p/M_s)^{1/2}$, and thus can be much larger than for near-resonant perturbations⁶. Below we describe briefly the dynamics of resonances that lead to these larger perturbations, and the conditions under which the putative planets may be in exact resonance.

Near a 3:2 commensurability of the orbital frequencies, n_1 and n_2 , there are technically two distinct first-order resonances. This splitting of the resonance arises from the fact that the critical frequency

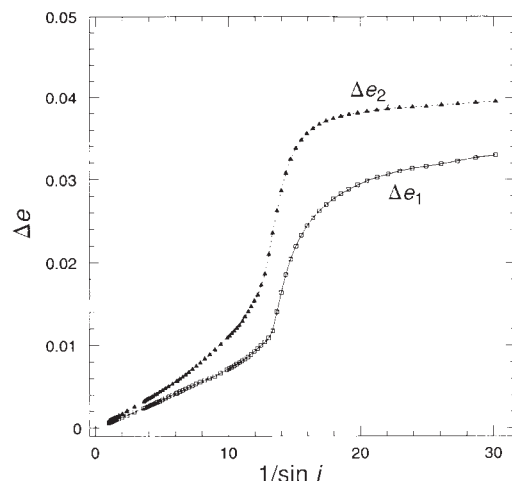
$$\nu = 3n_2 - 2n_1 \quad (1)$$

may be locked to the precession rate, $\dot{\omega}_i$, of either orbit. Thus, two resonance angles may be defined

$$\begin{aligned} \phi_1 &= 3\lambda_2 - 2\lambda_1 - \varpi_1 \\ \phi_2 &= 3\lambda_2 - 2\lambda_1 - \varpi_2 \end{aligned} \quad (2)$$

where λ_i are the instantaneous mean longitudes of the planets and ϖ_i are the longitudes of periastron. Exact resonance exists if the corresponding resonance angle is stationary or librates about a fixed value. Standard single resonance theory⁷ shows

FIG. 3 Eccentricities e_1 (—□—) and e_2 (---▲---) will change with time with an amplitude determined by the masses of the planets as well as the initial conditions. For the nominal orbital parameters in ref. 1, we show the maximum variation over a period of 5 years for different values of the factor $(\sin i)^{-1}$, as determined by a numerical integration of the equations of motion for the three-body system.



that the stable centre of libration for ϕ_1 is at 0, whereas for ϕ_2 it is at π . Depending upon the precise parameters of the system, it is possible for the planets to be locked in one or both resonances. Alternatively, the planets may not be locked in either resonance, in which case, over a period of time, the resonance angles will circulate through 2π radians. The difference between the two situations leads to significantly different dynamical behaviour of the orbital elements.

Perhaps the most easily detected signature of a resonance is the behaviour of the orbital eccentricity, e . Figure 1 shows the structure of the phase space trajectories in a plane on which the polar coordinates are (e_1, ϕ_1) or $(e_2, \phi_2)^{7-9}$. For given initial values $(e_{i,0}, \phi_{i,0})$ the motion of the planets will follow the closed curve passing through $(e_{i,0}, \phi_{i,0})$ in this plane. The bold curve designates the resonance separatrix which separates the (resonance) libration region of ϕ from regions where ϕ circulates. It is clear that on trajectories in the resonance zone, the eccentricity (distance from the origin) suffers large variations, whereas on trajectories outside the resonance region, the variations in e are much smaller. Furthermore, the resonant variations are, in general, complicated functions of time involving elliptic functions, unlike the simple time dependence of the near-resonant variations².

The location and width of the resonance region are functions of the masses and orbital radii of the planets^{6,9}. The location may be characterized by the centre of the resonance region (marked by an X in Fig. 1); its distance from the origin is given by

$$\begin{aligned} e_{1,\text{res}} &\approx \left(\frac{\nu}{6gn_1} \right)^{1/2} \\ e_{2,\text{res}} &\approx \left(\sqrt{\alpha} \frac{m_1}{m_2} \frac{\nu}{6gn_1} \right)^{1/2} \end{aligned} \quad (3)$$

for the ϕ_1 and ϕ_2 resonances, respectively. Here $\alpha = a_1/a_2$ is the ratio of the semimajor axes, and $g = 1 + (m_1/m_2)/\alpha$. (In the limit of the restricted three-body problem, this relationship between e and ν is an expression of the conservation of the Jacobi integral at the 3:2 resonance.) Note that $e_{i,\text{res}}$ is sensitive only to the orbital semimajor axes and the ratios of the masses, both of which are apparently known accurately. For the reported parameters of the system¹, the numerical values are $e_{1,\text{res}} \approx 0.046 \pm 0.005$ and $e_{2,\text{res}} \approx 0.047 \pm 0.005$.

The resonance width may be characterized by the minimum and maximum values of the orbital eccentricity on the resonance separatrix. These are sensitive to the magnitudes of the masses, that is, to the factor $(\sin i)^{-1}$. In Fig. 2, we show e_{min} and e_{max} as a function of $(\sin i)^{-1}$.

The observed eccentricities are 0.022 ± 0.007 and 0.020 ± 0.006 , respectively. Therefore, from Fig. 2, we infer that for $(\sin i)^{-1} \leq 10$ the planets are outside the resonance region. For $(\sin i)^{-1} \geq 10$, precise values of the complete set of orbital elements will determine whether the planets are locked in a resonance. For those initial conditions that place the system in the resonance zone, the eccentricity will oscillate about the mean value given in equation (3), over a characteristic resonance libration period¹⁰ which is proportional to $(m_p/M_s)^{-1/2}$ and therefore scales as $(\sin i)^{1/2}$; the libration period is ~ 12 years for $(\sin i)^{-1} \approx 10$.

For the nominal values of orbital elements inferred from ref. 1, Fig. 3 shows the expected variations of the eccentricities over 5 years for different values of the mass factor $(\sin i)^{-1}$. The observed orbital elements are favourable for resonance locking if $(\sin i)^{-1} \geq 10$. We also note that the condition for the existence of both ϕ_1 and ϕ_2 resonances requires that the difference between the resonance angles, or, equivalently, the difference between the longitudes of periastron, librates about π . It is suggestive that the observed difference¹ is close to π .

Finally, it should be noted that for random orientations of orbit planes, the probability that $(\sin i)^{-1} \geq 10$ is only about

0.5%. But with a sample of only one system, it is proper to consider the full range of possibilities. The existence of an exact resonance would have important implications for the formation and evolution of the planetary system. $\square$

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Photoconductivity of fullerene-doped polymers

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PHOTOCONDUCTING materials are employed in many technological applications, such as photodetection and electrostatic imaging.¹ We report here the preparation of photoconducting films of polyvinylcarbazole (PVK) doped with fullerenes (a mixture of C₆₀ and C₇₀). The performance of this material is comparable with some of the best photoconductors available commercially, such as thiapyrylium dye aggregates². The quantum yield for primary charge separation and the initial ion-pair distance are calculated, within the framework of the Onsager model^{3,4}, to be 0.9 and 19 Å respectively. The wavelength dependence of photoconductivity is essentially determined by the absorption spectrum of the fullerenes, with the active range extending from about 280 to 680 nm. The isolation of other fullerenes and fullerene derivatives may lead to the development of a large number of fullerene-based polymeric photoconductors in the future.

The fullerenes used were synthesized according to the electric arc method⁵, giving a mixture of C₆₀ and C₇₀ in the ratio $\sim 85:15$. Thin films of the fullerene-doped polymer are prepared by spin-coating fullerene and PVK (molecular weight $\sim 5 \times 10^5$) from solution in toluene (typically 40 mg of fullerene and 1.5 g of PVK in 12 ml of toluene) onto aluminium substrates. The samples are dried in an oven at 100 °C for several hours before use. The film thickness can be varied from 1 to 30 μm , by changing the concentration of PVK and the spin speed. The films are optically clear and air-stable for at least several months.

Photoconductivity is measured using the standard photo-induced discharge method^{6,7}. The sample film, deposited on an electrically grounded aluminium substrate, is first corona-charged (positively or negatively) in the dark. The amount of surface charge is detected by an electrostatic voltmeter (Monroe Electronics, model 244). On exposure to light, the photogenerated electrons and holes migrate to the surface, recombine with surface charges and discharge the surface potential. This photo-induced discharge process is the basis of xerography.

For light of sufficiently low intensity, absorbed within a small fraction of the film thickness, the charge generation efficiency, ϕ , can be obtained from the initial discharge rate of the surface potential, $(dV/dt)_{t=0}$ (refs 6, 7)

$$\phi = - \frac{\epsilon}{4\pi eLI} \left(\frac{dV}{dt} \right)_{t=0}$$

where ϵ is the dielectric constant, e the electronic charge, L the film thickness and I the absorbed photon flux. I have determined the wavelength and field dependence of ϕ using a xenon lamp as the light source below 400 nm, and a tungsten lamp for wavelengths >400 nm. The wavelength was selected using a monochromator. A Uniblitz shutter was used to limit the exposure time to 0.1 or 0.3 s. The light intensity used (typically 5×10^{12} – 6×10^{13} photons $\text{cm}^{-2} \text{s}^{-1}$) was low enough to avoid the space charge effect.

Figure 1 compares the photo-induced discharge curve of pure PVK with that of a fullerene-doped PVK film under identical experimental conditions. Broad band light from a tungsten lamp (50 mW cm^{-2}) was used in this case. This illustrates qualitatively the marked enhancement in the photo-induced discharge rate when PVK is doped to a few per cent with the fullerene mixture. Wavelength-dependent measurements show that the charge generation efficiency is enhanced by a factor of >50 at 500 nm and a factor of ~ 4 at 340 nm by doping PVK with $\sim 2.7\%$ fullerene (by weight). Figure 1 also shows that the fullerene-doped PVK film possesses low dark conductivity, and a photo-induced discharge that is both fast and complete; three important criteria for electro-photographic applications. I have compared it with polycarbonate film doped with thiapyrylium dye aggregate, one of the best commercial photoconductors², and found that both have comparable photo-induced discharge responses over a 40-fold change in the tungsten light intensity.

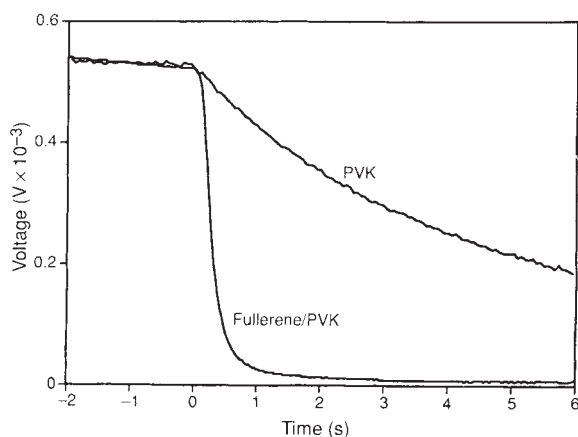


FIG. 1 A qualitative comparison of the photo-induced discharge curves for pure PVK and fullerene-doped PVK under the same experimental conditions. A tungsten lamp (50 mW cm^{-2}) is used as the light source.

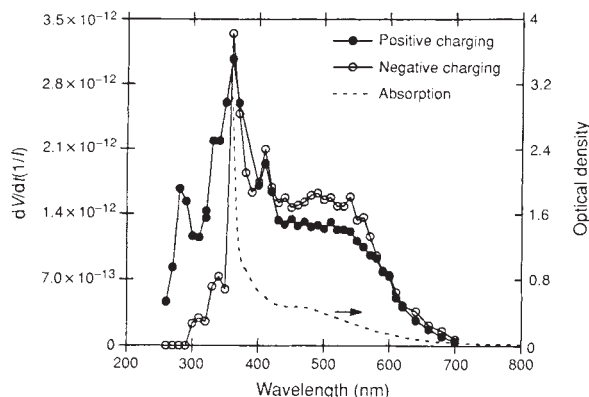


FIG. 2 The wavelength dependence of the charge generation efficiency for a $28 \mu\text{m}$ fullerene/PVK film, obtained for both positive (●) and negative (○) charging. The dashed line shows the optical absorption spectrum of the film. The applied voltage is $\sim 3 \times 10^5 \text{ V cm}^{-1}$.

Figure 2 shows the wavelength dependence of the charge generation efficiency ($(dV/dt)/(1/I)$), also called the gain spectrum) for a fullerene/PVK film with both positive and negative charging. Also shown is the optical absorption spectrum of the film. The gain spectra are similar to, but not identical with, the absorption spectrum, the visible region of the gain spectrum being relatively stronger than that of the absorption spectrum. For wavelengths above 350 nm, efficient photo-induced discharge can be achieved with both positive and negative charging, but in the strongly absorbing region (<350 nm), only positive charging is effective. For the strongly absorbing wavelengths, carriers are generated near the surface and have to migrate across the polymer film; the results show that only holes can achieve this.

Figure 3 shows the field dependence of the charge generation efficiency, measured at 340 nm with a photon flux of 1.62×10^{13} photons $\text{cm}^{-2} \text{s}^{-1}$. The Onsager model³ is recognized as a good starting point for analysing such data, although a modified version may be required for a full interpretation⁵. It provides a means by which the charge-generation efficiency of a range of photoconductors can be compared. The model assumes that the absorption of a photon creates a bound electron-hole pair with a thermalized separation distance of r_0 . This bound electron-hole pair either recombines, or separates into a free electron and hole. The fraction of absorbed photons that generate bound electron-hole pairs is the primary quantum yield, ϕ_0 , and is assumed to be field-independent. By fitting the experimental data to the Onsager model,^{3,4} I obtain values for both r_0 and ϕ_0 . Figure 3 shows the best-fit theoretical curve, giving $r_0 = 19 \text{ Å}$ and $\phi_0 = 0.9$. For comparison, I also show curves calculated using $r_0 = 17 \text{ Å}$ and $r_0 = 21 \text{ Å}$. The thermalization length and primary quantum yield of thiapyrylium dye aggregate films² are 44 Å and 0.58 respectively.

The effects of the fullerene on the charge generation and charge transport processes in this new photoconductor still need to be studied in detail. The fullerenes are clearly responsible for the charge generation, as demonstrated by the similarity between the gain spectra and the absorption spectrum (Fig. 2). I have also shown that fullerene can form charge-transfer (CT) complexes with electron-donating aromatic amines such as *N,N*-diethylaniline⁹. The charge-generating process could thus be due to either the excitation of fullerene (followed by electron hopping from carbazole to fullerene), or the direct excitation of fullerene/carbazole CT complexes. I cannot distinguish between these two possibilities using the gain spectra alone, as

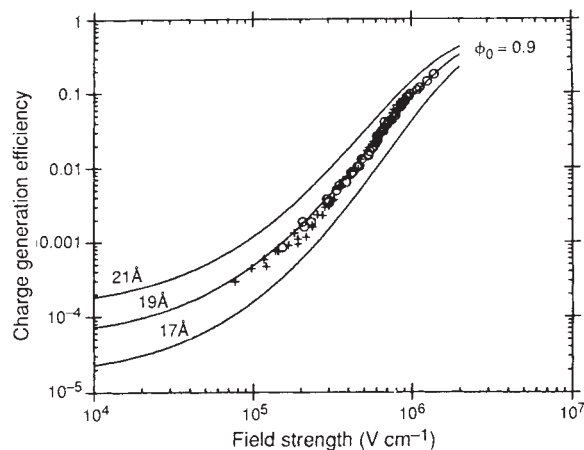


FIG. 3 The field dependence of the charge generation efficiency for $4.5 \mu\text{m}$ (○) and $9.3 \mu\text{m}$ (+) fullerene/PVK films, with positive charging and 340 nm irradiation. The solid lines are calculated from the Onsager model. The best-fit curve is obtained with $r_0 = 19 \text{ Å}$ and $\phi_0 = 0.9$.

both the C₆₀/carbazole CT complex and C₆₀ have similar absorption spectra: in a separate experiment, I found that the absorption spectrum of the C₆₀/carbazole CT complex differ from that of C₆₀ only by an enhanced, broad absorption band in the 500 nm region.

Because the concentration of fullerene used is only a few percent by weight (below the percolation threshold), the transport of carriers has to occur through the PVK polymer matrix. To understand the transport mechanism, it is important to study the effects of dopant level on the charge-carrier mobility. The doping level used in this study is near the maximum imposed by the solubility of fullerene in toluene. To increase the dopant level significantly, a different solvent is required. Increasing the dopant level should further enhance the photoconductive properties through enhanced light absorption and charge-generation efficiency, but any effect on the carrier mobility remains to be determined.

With the fullerene mixtures used in this work (~85% C₆₀), the photoconductor is sensitive only up to wavelengths ~680 nm. It has been reported that some higher fullerenes (for example, C₇₆) have absorption bands in the longer wavelength region^{10,11}. It is therefore possible that the range of sensitivity can be extended to the technologically important red and infrared regions using the higher fullerenes or fullerene derivatives. □

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Interhemispheric transport of carbon dioxide by ocean circulation

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ALTHOUGH anthropogenic emissions of carbon dioxide have today created a greater atmospheric CO₂ concentration in the Northern than in the Southern Hemisphere, a comparison of interhemispheric CO₂ profiles from 1980 and 1962 led Keeling and Heimann^{1,2} to conclude that, before the Industrial Revolution, natural CO₂ sources and sinks acted to set up a reverse (south to north) gradient which drove about one gigatonne of carbon each year through the atmosphere from the Southern to the Northern Hemisphere. At steady state, this flux must have been balanced by a counter flow of carbon from north to south through the ocean. Here we present a means to estimate this natural flux by a separation of oceanic carbon anomalies into those created by biogenic processes and those created by CO₂ exchange between the ocean and atmosphere. We find that before the Industrial Revolution, deep water formed in the northern Atlantic Ocean carried about 0.6 gigatonnes of carbon annually to the Southern

Hemisphere, providing support for Keeling and Heimann's proposal. The existence of this oceanic carbon pump also raises questions about the need for a large terrestrial carbon sink in the Northern Hemisphere, as postulated by Tans *et al.*³, to balance the present global carbon budget.

During the 1980s, the CO₂ content of the atmosphere at high northern latitudes was on average greater than that at southern high latitudes by ~3 p.p.m. (refs 1-3). This difference is only about half that predicted on the basis of model calculations which include CO₂ uptake by the southern oceans. To resolve this discrepancy, Tans *et al.*³ proposed that CO₂ is being efficiently sequestered into a temperate, high-latitude terrestrial reservoir, implying that anthropogenic CO₂ (or nitrate) is acting as a potent global plant fertilizer in this region (although not in the tropics). They also concluded that the oceans were not taking up anywhere near the amount of CO₂ predicted from ocean models. The hypothesis of Keeling and Heimann^{1,2} that natural carbon sources and sinks produce a south-to-north gradient in atmospheric CO₂ might, however, negate the need to invoke a terrestrial sink of this sort.

The Atlantic Ocean's conveyor circulation⁴ is the most likely candidate for interhemispheric CO₂ transport because it carries to the Southern Hemisphere, at a rate of $2 \times 10^7 \text{ m}^3 \text{ s}^{-1}$, water that was in contact with the Northern Hemisphere atmosphere before sinking into the deep sea⁵. If, before the Industrial Revolution, the water moving northward in the upper Atlantic carried less dissolved carbon than this southward-flowing deep water, then the conveyor could have acted as an interhemispheric carbon pump. If this pump is to transport a gigatonne of carbon, then the southward-flowing deep water would have to carry ~130 $\mu\text{mol kg}^{-1}$ (or ~6%) more dissolved carbon than the northward-flowing upper water.

We have come up with a means of estimating the magnitude of this difference. The amount of ΣCO_2 ($\text{HCO}_3^- + \text{CO}_3^{2-} + \text{CO}_2$) contained in a given parcel of water is compared with the amount that the parcel would contain if it had neither gained CO₂ from the atmosphere nor lost CO₂ to the atmosphere; in other words, with that which would be present were no exchange of CO₂ between ocean and atmosphere to occur. The difference, $\Delta \Sigma \text{CO}_2$, between observed and expected ΣCO_2 is then a measure of the amount of CO₂ gained from or lost to the atmosphere by a given water parcel. To calculate the expected ΣCO_2 concentration, account must be taken of the addition or removal of fresh water (based on salinity, *S*), soft-tissue carbon (based on phosphate) and CaCO₃ (based on nitrate-corrected alkalinity). The equation we use is

$$\Sigma \text{CO}_2^{\text{EXP}} = 1,900 \times \frac{S}{35.00} + 127 \text{ PO}_4 + \frac{1}{2} \left(\text{Alk} + \text{NO}_3 - 2,310 \frac{S}{35.00} \right)$$

where 1,900 $\mu\text{mol kg}^{-1}$, 2,310 $\mu\text{equiv kg}^{-1}$ and 35.00 g l^{-1} are arbitrary constants designed to bring the average value of $\Delta \Sigma \text{CO}_2$ for the ocean close to zero. The constant 127 is our best estimate of the average carbon/phosphorus ratio in marine organic matter⁶⁻⁸.

One further correction must be made: because of the uptake of fossil-fuel CO₂, the ΣCO_2 content of the upper waters of the ocean is higher than it was before the Industrial Revolution. We deal with this complication as follows. For surface waters, we calculate the equilibrium ΣCO_2 increase expected if the water parcel had come to equilibrium with the atmosphere CO₂ excess (at the time that the sample was collected). We multiply this amount by 0.85 and subtract it from the observed value. The 0.85 multiplier is based on results from tracer-calibrated models of the ocean-atmosphere carbon cycle which show that the resistance to fossil-fuel CO₂ uptake posed by mixing within the sea is about seven times larger than that posed by the air-sea boundary. The fossil-fuel CO₂ correction is ~40 $\mu\text{mol kg}^{-1}$ on

average and should be accurate to $\pm 10 \mu\text{mol kg}^{-1}$. For deep waters well away from their surface source regions, we assume that no fossil-fuel CO_2 addition has occurred (so that the observed value is representative of pre-industrial conditions).

The distribution of fossil-fuel-corrected $\Delta \Sigma \text{CO}_2$ values for the world's surface waters is summarized in Fig. 1. The highest positive values are found in the northern Atlantic where the average fossil-fuel-corrected $\Delta \Sigma \text{CO}_2$ ranges from 75 to more than $100 \mu\text{mol kg}^{-1}$. The highest negative values are found in the tropics of the Pacific and Indian Ocean. The important point here is not the negative $\Delta \Sigma \text{CO}_2$ values in the tropics, for the loss to the atmosphere which created these deficiencies would not give rise to an interhemispheric gradient. Rather, it is the difference of $80 \mu\text{mol kg}^{-1}$ between the northern Atlantic and the Antarctic.

A similar difference is suggested by the data for deep waters in the Atlantic. These waters are mixtures of North Atlantic Deep Water (NADW) entering from the north, and bottom and intermediate waters of Antarctic origin entering from the south. An estimate of the contribution of these sources to any given water parcel is provided by a property PO_4^* which is defined as follows⁹:

$$\text{PO}_4^* = \text{PO}_4 - 1.95 + \frac{\text{O}_2}{175}$$

where 1.95 is an arbitrarily chosen constant and 175 is the average molar ratio of oxygen consumed to PO_4 released as a consequence of respiration in the deep sea^{6,7}. As the decrease in the O_2 contribution to PO_4^* should be balanced by an increase in PO_4 , this property is quasiconservative. Northern component waters have a PO_4^* value of 0.73 ± 0.03 and southern component waters a value of 1.67 ± 0.05 . Values in between are created by mixing between the endmember waters. As shown in Fig. 2, when plotted against PO_4^* , the $\Delta \Sigma \text{CO}_2$ values for deep Atlantic waters fall along two trends, the upper trend for deep waters and the lower trend for intermediate waters. Deep waters of northern origin have higher $\Delta \Sigma \text{CO}_2$ values than those of southern origin. The $\Delta \Sigma \text{CO}_2$ values for northern-component

water range from $+80$ to $+115 \mu\text{mol kg}^{-1}$. This range is consistent with the fossil-fuel-corrected values for northern Atlantic surface waters. The value for southern component water ($+25 \mu\text{mol kg}^{-1}$) agrees well with the fossil-fuel-corrected average for Antarctic surface waters ($+19 \mu\text{mol kg}^{-1}$). As for surface waters corrected for fossil-fuel CO_2 , we find that the difference between the northern Atlantic and Antarctic derived water is $\sim 80 \mu\text{mol kg}^{-1}$.

But account must be taken of the fact that the main component of the return flow balancing the export of NADW is not at deep or intermediate depths. Rather, it is in the upper ocean. Fortunately, surface waters in the Drake Passage, in the Agulhas retroflection around the tip of Africa and in the Bering Sea have $\Delta \Sigma \text{CO}_2$ values corrected for fossil-fuel CO_2 that are close to the average for Antarctic surface water (see Fig. 1). Regardless of the proportions transported along these various pathways, therefore, the difference in $\Delta \Sigma \text{CO}_2$ between the water exported via the lower limb of the conveyor and that imported by the upper limb should be $\sim 80 \mu\text{mol kg}^{-1}$. Current meter measurements¹⁰, geostrophic flow calculations¹¹ and radiocarbon measurements⁵ all suggest that transport by the Atlantic's conveyor is ~ 20 Sverdrups. Coupled with the difference of $80 \mu\text{mol kg}^{-1}$ between the $\Delta \Sigma \text{CO}_2$ values for upper and lower conveyor water, this leads to an interhemispheric transport of 0.6 gigatonnes of carbon per year.

What is responsible for the $\Delta \Sigma \text{CO}_2$ difference between northern Atlantic and Antarctic surface water? The answer lies in the difference between the PO_4 contents of surface waters in these regions; the average for the Antarctic is $1.7 \mu\text{mol kg}^{-1}$ and that for northern Atlantic is $0.9 \mu\text{mol kg}^{-1}$. The difference of $0.8 \mu\text{mol kg}^{-1}$ corresponds to $\sim 100 \mu\text{mol kg}^{-1}$ of respiration CO_2 . If uncompensated by transport through the atmosphere, this extra CO_2 would lead to much higher CO_2 partial pressures in Antarctic than in northern Atlantic surface waters; the average for the Antarctic would be $\sim 280 \mu\text{atm}$ and that for the northern Atlantic $\sim 160 \mu\text{atm}$ (see Fig. 3). In the real world, CO_2 exchange between air and sea occurs fast enough to eliminate much of the difference in CO_2 partial pressure among surface waters. Warm tropical waters tend to give up CO_2 to the atmosphere and cold polar waters tend to absorb CO_2 from the atmosphere. But because of their high respiration CO_2 content, even without transport through the atmosphere, Antarctic surface waters would have CO_2 partial pressures roughly in balance with warm surface waters (see Fig. 3). The extra respiration CO_2 is just

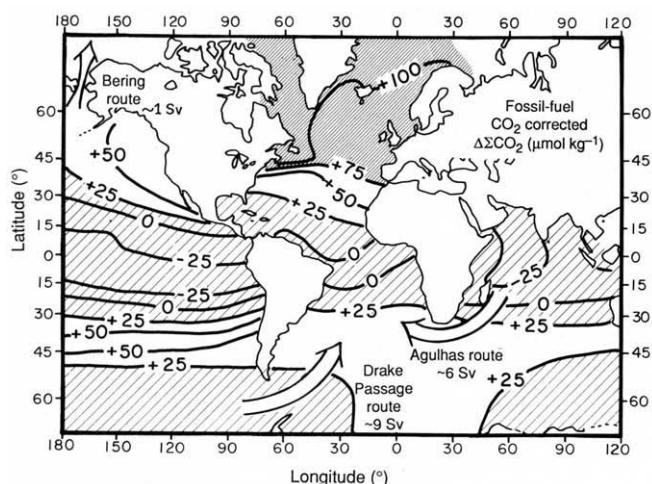


FIG. 1 Map showing the geographical distribution $\Delta \Sigma \text{CO}_2$ values (corrected for fossil-fuel CO_2) for surface waters based on measurements of salinity, PO_4 , NO_3 , ΣCO_2 , and alkalinity made during the GEOSECS and TTO expeditions¹³⁻¹⁶. In the temperate North Atlantic where geographical overlap exists between these two expeditions, the data sets are concordant. Calculations from measurements on surface waters in the Atlantic north of Iceland during the Hudson-1982 winter expedition¹⁷ yield values of $\Delta \Sigma \text{CO}_2$ similar to those obtained during the TTO-1981 summer expedition¹⁶. The return routes for water, balancing export from the Atlantic via the conveyor's lower limb, are shown by arrows, with rough estimates of the magnitude of their contributions in Sverdrups ($10^6 \text{ m}^3 \text{ sec}^{-1}$). Northward-flowing deep water contributes the remaining four Sverdrups.

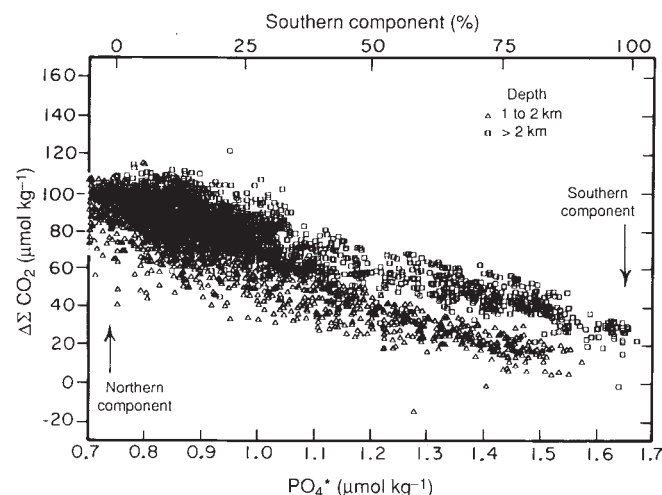


FIG. 2 $\Delta \Sigma \text{CO}_2$ against PO_4^* for intermediate waters (Δ) and for deep waters ($\square$) in the Atlantic Ocean (40°N to 40°S). PO_4^* is a measure of the contribution of Antarctic-derived waters to any given water parcel. The PO_4^* and $\Delta \Sigma \text{CO}_2$ values are calculated from measurements made as part of the GEOSECS¹³⁻¹⁵, TTO¹⁶, TAS¹⁸ and SAVE¹⁹ expeditions in the Atlantic Ocean.

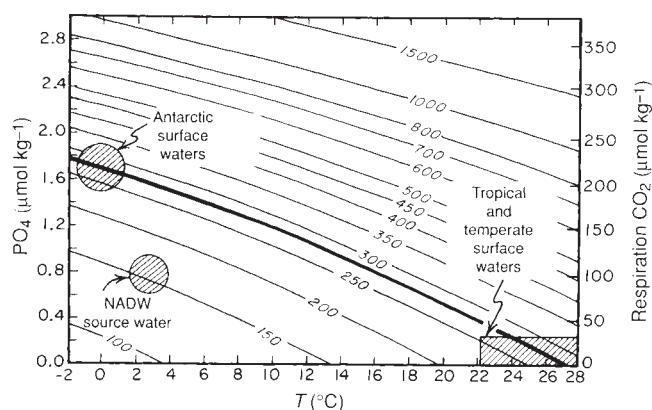


FIG. 3 Contours of CO_2 partial pressure (in μatm) for a water sample with a salinity of 35‰, a respiration free ΣCO_2 concentration of $1,900 \mu\text{mol kg}^{-1}$ and an alkalinity of $2,310 \mu\text{equiv kg}^{-1}$ as a function of water temperature and phosphate content (and hence also respiration CO_2 content). We assume a C/P ratio of 127 and a N/P ratio of 16 for the marine organic matter oxidized within the sea. The heavy contour is that representing the pre-industrial atmosphere ($280 \mu\text{atm}$). Note that in the real ocean, fresh-water-induced salinity and CaCO_3 -induced alkalinity differences create additional texture in the distribution of CO_2 partial pressure in surface water. The important point here is that the high content of respiration CO_2 compensates for the low temperature of Antarctic surface waters, giving them nearly the same CO_2 partial pressure as warm nutrient-free surface water. By contrast, because of their lower respiration CO_2 content, surface waters in the northern Atlantic have much lower CO_2 pressures. As a consequence, surface waters in the northern Atlantic take up CO_2 given off to the atmosphere by other regions of the ocean.

enough to compensate for their lower temperature. By contrast, because of their lower content of respiration CO_2 , surface waters in the northern Atlantic have a strong tendency to take up CO_2 from the atmosphere. Thus it is the difference in respiration CO_2 content which creates the tendency to pump CO_2 through the ocean from north to south and through the atmosphere from south to north.

Our estimate of 0.6 gigatonnes for the natural transport of carbon through the ocean from the Northern to the Southern Hemisphere can be compared with that of 0.26 gigatonnes obtained by Brewer *et al.*¹² for the net southward transport across a section at 25°N in the Atlantic. As Brewer *et al.* did not correct for fossil-fuel CO_2 carried northward by the upper waters, their estimate provides only the lower limit on the pre-industrial interhemispheric transport.

According to our calculations, transport of excess CO_2 by the Atlantic's conveyor accounts for $\sim 60\%$ of the mismatch between the amount of CO_2 currently being transported across the Equator and the amount required to explain the increases observed for the Southern Hemisphere atmosphere and calculated for the Southern Hemisphere ocean. The 0.6 gigatonnes of CO_2 naturally released from the southern ocean offsets part but not all of the one gigatonne uptake of anthropogenic CO_2 calculated by oceanographers. Thus, although it does not invalidate the case made by Tans *et al.*³ for a reduced CO_2 uptake by the ocean and an increased CO_2 uptake by the north temperate terrestrial biosphere, our finding removes much of the punch of this argument. It also lends support to the proposal by Keeling and Heimann^{1,2} that a south-to-north decrease in atmospheric CO_2 content existed before the Industrial Revolution. The challenge will be to document this small difference directly through ultra-precise measurements of CO_2 to air ratios in pre-industrial ice from Antarctica and from Greenland. □

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Revised budget for the oceanic uptake of anthropogenic carbon dioxide

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TRACER-CALIBRATED models of the total uptake of anthropogenic CO_2 by the world's oceans give estimates of about 2 gigatonnes carbon per year¹, significantly larger than a recent estimate² of $0.3\text{--}0.8 \text{ Gt C yr}^{-1}$ for the synoptic air-to-sea CO_2 influx. Although both estimates require that the global CO_2 budget must be balanced by a large unknown terrestrial sink, the latter estimate implies a much larger terrestrial sink, and challenges the ocean model calculations on which previous CO_2 budgets were based. The discrepancy is due in part to the net flux of carbon to the ocean by rivers and rain, which must be added to the synoptic air-to-sea CO_2 flux to obtain the total oceanic uptake of anthropogenic CO_2 . Here we estimate the magnitude of this correction and of several other recently proposed adjustments to the synoptic air-sea CO_2 exchange. These combined adjustments minimize the apparent inconsistency, and restore estimates of the terrestrial sink to values implied by the modelled oceanic uptake.

Table 1a shows annual anthropogenic CO_2 budgets for recent years as summarized by the Intergovernmental Panel on Climate Change (IPCC)¹ and Tans *et al.*². Both budgets show large imbalances which are generally attributed to uptake by terrestrial vegetation and/or soils³, although firm evidence for this uptake is difficult to obtain and the uncertainties are therefore large. The IPCC budget was based on models of ocean CO_2 uptake which imply a net terrestrial carbon flux near zero (Table 1b). Tans *et al.* estimated the synoptic air-to-sea CO_2 flux by combining the results of an atmospheric transport model with a compilation of observations of air-sea CO_2 difference. They concluded that the limited rate of atmospheric transport of anthropogenic CO_2 from the Northern Hemisphere restricts ocean CO_2 uptake south of the Equator, and the air-sea CO_2 observations set limits on oceanic CO_2 absorption in the Northern Hemisphere. They proposed that ocean CO_2 uptake constrained in this way must be $0.3\text{--}0.8 \text{ Gt C yr}^{-1}$, requiring a total net terrestrial uptake of $\sim 1.5\text{--}2.0 \text{ Gt C yr}^{-1}$ (Table 1b).

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TABLE 1 Budgets for anthropogenic perturbation of CO₂

(a) IPCC ¹ and Tans <i>et al.</i> ²		
	Average perturbation (Gt C yr ⁻¹)	
	IPCC*	Tans <i>et al.</i> †
Sources		
Fossil	5.4 ± 0.5	5.3
Deforestation	1.6 ± 1.0	0.0–3.2
Total	7.0 ± 1.2	5.3–8.5
Sinks		
Atmosphere	3.2 ± 0.1	3.0
Oceans (steady-state models)	2.0 ± 0.8	0.3–0.8
Total	5.2 ± 0.8	3.3–3.8
Imbalance (inferred terrestrial uptake)	1.8 ± 0.4	2.0–4.7
(b) Comparison of the IPCC terrestrial and oceanic sinks ¹ with the budget of Tans <i>et al.</i> ²		
	IPCC*	Tans <i>et al.</i> †
(1) Inferred terrestrial uptake	1.8 ± 1.4	2.0–4.7
(2) Deforestation	1.6 ± 1.0	0.0–3.2
Net terrestrial uptake, (1) – (2)	0.2 ± 1.7	1.5–2.0
Net ocean uptake	2.0 ± 0.8	0.3–0.8
Total uptake (terrestrial + ocean)	2.2 ± 1.9	2.3
(c) Modified IPCC ¹ and Tans <i>et al.</i> ² ocean sink budgets		
	Fluxes in Gt C yr ⁻¹	
Revised Tans <i>et al.</i> budget		
Tans <i>et al.</i> synoptic estimates of air–sea input	0.3–0.8	
Correction for skin temperature effect	0.1–0.6	
Correction for carbon monoxide budget	0.3	
Modified estimate of air–sea input	0.7–1.7	
Net river inorganic carbon flux	0.2–0.3	
Net river organic carbon flux	0.2–0.4	
Total ocean uptake	1.1–2.4	
Revised IPCC budget		
Model estimates of oceanic uptake	1.7–2.8	

* The IPCC budget covers the period 1980–89. The atmospheric sink has been reduced to 3.2 ± 0.1 from the original IPCC value 3.4 ± 0.2 (P. Tans, personal communication).

† The Tans *et al.* budget is based on their scenarios 5–8, which make use of an atmospheric transport model constrained by the interhemispheric gradient of CO₂ as estimated from observations for the period 1980–87, and by oceanic observations in the region between 15° S and 90° N for the period 1972–89. The 15° S–90° N oceanic observations give a global uptake of 0.35 Gt C yr⁻¹ with the gas exchange coefficient of ref. 9, and 0.71 Gt C yr⁻¹ with an empirical gas exchange coefficient based on observations of ocean uptake of bomb radiocarbon. The Tans *et al.* scenarios adjust the Southern Hemisphere ocean uptake so that the total ocean uptake with the two different gas exchange coefficients is comparable. The total terrestrial and ocean uptake is fixed at 2.3 Gt C yr⁻¹ in all their scenarios.

Such a small oceanic sink for anthropogenic CO₂ is inconsistent with many ocean models. For example, the work of Sarmiento *et al.*⁴, which makes use of a three-dimensional ocean model validated against bomb radiocarbon observations⁵, gives an estimate of 1.9 Gt C yr⁻¹ for the decade 1980–89 and 1.7 Gt C yr⁻¹ for the period 1972–89, which is the time span of the data set used by Tans *et al.*². Sarmiento *et al.* conclude that these estimates probably represent a lower limit; the ocean circulation model underestimates the bomb radiocarbon uptake because of factors that would also underestimate the uptake of anthropogenic CO₂. We therefore suggest 1.7 Gt C yr⁻¹ as a lower limit for the modelled oceanic uptake of anthropogenic CO₂ while retaining the IPCC upper limit of 2.8 Gt C yr⁻¹ (Table 1c).

Although the air–sea CO₂ flux estimates of ref. 2 are based on the most comprehensive available data set, they may not

TABLE 2 Estimated marine and terrestrial pre-industrial carbon budgets

(a) Geochemical ocean inorganic carbon budget*				
	Flux (Gt C yr ⁻¹)			
	Berner <i>et al.</i> ²³	Berner and Berner ²⁴	Meybeck ²⁵	Drever <i>et al.</i> ²⁶
(1) River dissolved inorganic carbon	0.429	0.390	0.439	0.385
(2) Marine carbonate sedimentation	0.221	0.204	0.236	0.127–0.170
(1) – (2)	0.208	0.186	0.203	0.215–0.258
Decarbonation	0.071	0.080	0.077	—
Implied ocean–atmosphere flux of CO ₂	0.21–0.28	0.19–0.27	0.20–0.28	0.21–0.26
(b) Recent estimates of global river organic carbon fluxes				
	Flux estimates (Gt C yr ⁻¹)			
Reference	Dissolved organic carbon	Particulate organic carbon	Total organic carbon	
Schlesinger and Melack ²⁷	—	—	0.37	
	—	—	0.41	
Meybeck ²⁸	0.215	0.179	0.383	
Milliman <i>et al.</i> ²⁹	—	(0.295)†	—	
Ittekkot ³⁰	—	0.231	—	
Meybeck ³¹	0.206	0.169	0.302	
			(0.375)‡	
Spitzzy and Leenheer ³²	0.22	—	—	
Degens <i>et al.</i> ³³	—	—	0.33	
			(0.365)§	
'Best' estimates	0.2	0.2–0.3	0.3–0.5	
(c) Estimated terrestrial and marine organic carbon budgets				
	Flux (Gt C yr ⁻¹)			
Oceans				
Additions by rivers			0.3–0.5	
Burial in sediments			–0.1	
Net loss to CO ₂			0.2–0.4	
Land				
Production delivered to rivers			–0.3––0.5	
Net oxidation or organic carbon in rocks			0.1	
Net production from CO ₂			–0.2––0.4	

* The estimates of refs 23–25 are all based on the assumption of a steady state with stoichiometry similar to the reactions in Fig. 1a and b. A small correction was applied by the authors of these estimates to account for weathering by H₂SO₄ from pyrite oxidation on land, balanced by alkalinity production during sulphate reduction in marine sediments. These processes result in a bicarbonate river flux to the ocean, balanced by an equal amount of CaCO₃ burial in the sediments, and explain why row (2) is more than 50% of row (1). The estimates of ref. 26 were not constrained to a steady state and did not include an estimate of silicate weathering and decarbonation.

† Based on the revision suggested in ref. 29 of the particulate organic carbon flux estimates of Meybeck²⁸.

‡ Calculated as sum of dissolved organic carbon and particulate organic carbon fluxes; disagreement with the total organic flux estimate of ref. 31 is due to extrapolation from different data sets.

§ The estimate of ref. 33 for the total organic carbon flux for Asia is biased towards the relatively low ratios of total organic carbon to discharge measured in the rivers flowing into the Arctic Ocean. Using their total organic carbon data and method of extrapolation, but extrapolating separate estimates for northern and southern Asian rivers, we calculate an increase of 18% (from 0.169 to 0.200 Gt C yr⁻¹) in the aggregate extrapolated total organic carbon flux from Asia.

|| The maximum estimate is calculated as the sum of the maximum 'best' estimates for dissolved organic carbon and particulate organic carbon.

accurately represent average annual air–sea CO₂ differences. We doubt, however, that improved temporal and spatial resolution could account for a systematic Northern Hemisphere flux error in excess of 1 Gt C yr⁻¹, which would be required to reconcile the results of ref. 2 with the ocean model results. The difficulty can be illustrated by comparing air–sea CO₂ differences calculated by Tans *et al.* with those derived from a recent model

study⁶, which includes the interhemispheric transport constraint discussed by Tans *et al.*, but ignores the estimates of air-sea CO₂ flux in the Northern Hemisphere, using instead the uptake of 2.3 Gt C yr⁻¹ estimated for 1984 from an oceanic model. This model requires an air-sea CO₂ difference of +19 p.p.m. in the Pacific north of 15.6° N, compared with an estimate of -2 p.p.m. based on the data of Tans *et al.*, and an air-sea difference of +52 p.p.m. north of 23.5° N in the Atlantic, compared with data-based estimates by Tans *et al.* of +37 p.p.m. north of 50° N and +15 p.p.m. between 15° and 50° N.

An important factor not accounted for by Tans *et al.*² is the observation that skin temperatures of water at the surface of the ocean are usually colder than the bulk temperatures normally used in determining air-sea pCO₂ differences. One recent study⁷ in the North Atlantic showed that surface temperatures were ~0.11 °C colder than bulk temperatures at day, on average, and ~0.3 °C colder during the night.⁷ The solubility of CO₂ in water increases by 3–4% per °C cooling, with the result that the cooler skin temperatures would give an increase in the average of data-based air-sea CO₂ difference estimates of ~1.1–4.3 p.p.m. (see, for example, Smethie *et al.*⁸). This correction could have a considerable effect on the global carbon budgets (A. Watson, personal communication). An increase of this magnitude in the air-sea CO₂ difference over the entire globe would give an increased flux of CO₂ into the ocean of 0.14–0.54 Gt C yr⁻¹, assuming a mean global gas exchange coefficient of 0.029 mol m⁻² yr⁻¹ per p.p.m. (ref. 9). An average gas exchange coefficient high enough to explain the bomb radiocarbon observations (this would be 0.061 mol m⁻² yr⁻¹ per p.p.m.; see ref. 5), would give an increase in the observationally based ocean

uptake estimate of 0.29–1.12 Gt C yr⁻¹. Tans *et al.*² made use of ocean observations only for the region north of 15° S (57% of the ocean area), so the total range of these estimated corrections (0.14 to 1.12) would have to be multiplied by ~0.57, giving 0.1–0.6 Gt C yr⁻¹, to account for the skin temperature effect (Table 1c).

Another correction is for the effect of carbon monoxide transport and oxidation on the CO₂ budget. Carbon monoxide is produced mainly in the Northern Hemisphere by industrial and biotic processes¹⁰. It is oxidized to CO₂ primarily by reaction with hydroxyl in the atmosphere both north and south of the Equator. These sources and sinks effectively mediate the transport of 0.25–0.29 Gt C yr⁻¹ of fossil-fuel CO₂ to the Southern Hemisphere as atmospheric carbon monoxide¹¹. Tans *et al.* did not include this transfer in their calculations, although they did briefly mention it. We assume that the main sink for this carbon monoxide-mediated interhemispheric CO₂ transport is the Southern Hemisphere ocean, and include it as a correction to the Tans *et al.* oceanic uptake shown in Table 1c.

Geochemists have long postulated a net CO₂ flux associated with the river flux of carbon into the ocean. Its possible contribution to the anthropogenic CO₂ budget has been noted before (see, for example, ref. 12), but was not included in the oceanic uptake estimated in ref. 2, nor were its full implications noted in ref. 12 for the comparison between the Tans *et al.* budget and the ocean model estimates of anthropogenic CO₂ uptake. Even if there were no anthropogenic CO₂ source, the river carbon flux must be geochemically balanced by a small but important long-term net CO₂ flux from the oceans to the atmosphere.

The dominant geochemical fluxes affecting CO₂ are those

FIG. 1 Illustration of the effect of the carbonate, silicate and organic carbon budgets on air-sea exchange of CO₂. M symbolizes a generic cation such as Ca²⁺. The thick arrows indicate the actual fluxes that are summarized in Table 2. *a*, Carbonate budget. The flux of bicarbonate into the ocean by rivers is balanced by a 50% loss of CO₂ to the atmosphere and a 50% loss of MCO₃ to the sediments. The ocean-to-atmosphere flux can be estimated from the river bicarbonate flux minus the sediment burial of MCO₃. *b*, Silicate budget. Again, this implies a CO₂ flux that can be estimated by subtracting the burial of MCO₃ from the river flux of bicarbonate, as in the carbonate budget. The volcanic and metamorphic decarbonation reaction releases additional CO₂ which must enter the atmosphere to close the budget, and can do so either through the ocean or directly to the atmosphere. The decarbonation flux is estimated from the silicate weathering flux and is treated as an uncertainty, as discussed in the text. *c*, Organic carbon budget. This has two parts: the terrestrial burial and riverine export of photosynthetic carbon (left) requires a net terrestrial uptake of CO₂ which must be met by a flux out of the ocean; the oceanic burial of photosynthetic carbon must be balanced by a net oxidation on land (right), which results in CO₂ uptake by the ocean. At steady state, the net air-sea exchange of CO₂ must be balanced by all of these processes, among which the riverine organic flux predominates. One can therefore estimate the effect of the organic carbon budget on air-sea exchange from the river flux of organic carbon minus the ocean sediment burial of organic carbon.

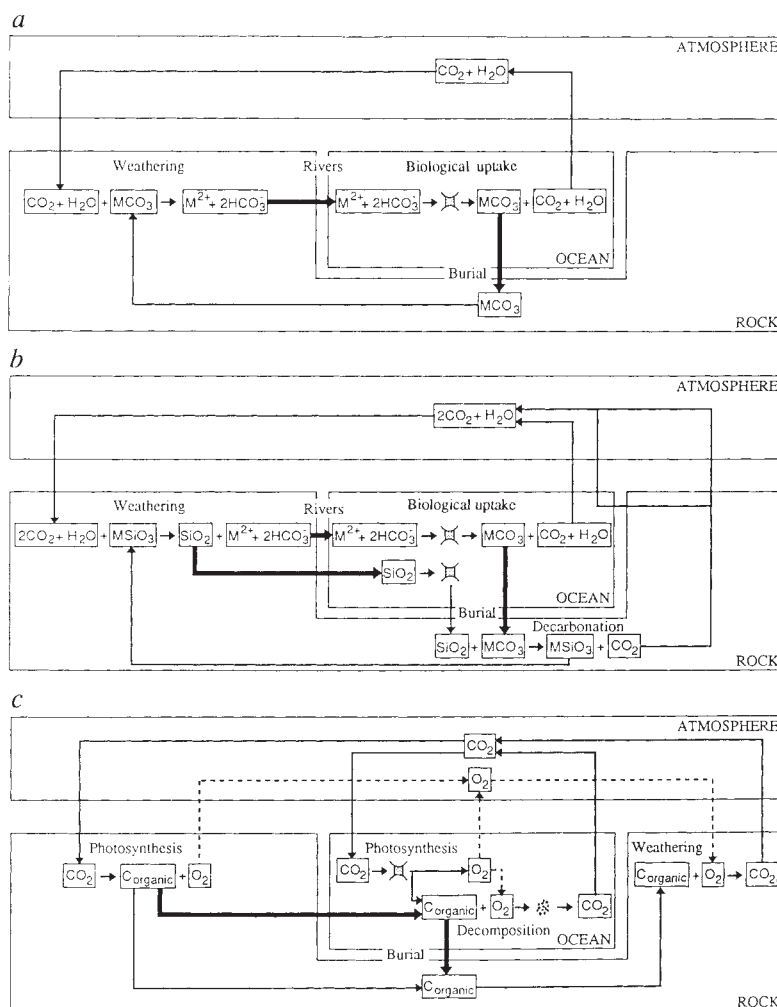


TABLE 3 Spatial distribution of ocean to atmosphere CO₂ flux

Latitude band	A	B	C = A - B
	Revised Tans <i>et al.</i> ² budget*	Anthropogenic CO ₂ uptake estimate†	Estimated pre-industrial fluxes
>15°N	-0.66	-0.38	-0.28
15°S-15°N	1.22	-0.48	+1.70
15°S-50°S	-2.15	-0.41	-1.74
>50°S	0.50	-0.42	+0.92
Total	-1.09	-1.69	+0.60

* The Tans *et al.*² budget is based on their scenario 6. This scenario gives an ocean uptake of $-0.69 \text{ Gt C yr}^{-1}$ which needs to be increased to $-1.09 \text{ Gt C yr}^{-1}$ to match the pre-industrial ocean efflux estimate of 0.6 Gt C yr^{-1} shown in column C, added to the anthropogenic CO₂ uptake estimate of column B. This is accomplished by adding the $-0.25 \text{ Gt C yr}^{-1}$ carbon monoxide correction obtained by Enting and Mansbridge¹¹ to the 15°S-50°S band, and by adding a further $-0.15 \text{ Gt C yr}^{-1}$ skin-temperature correction split evenly between the equal areas of the equatorial and Northern Hemisphere bands. The reason for putting all of the Southern Hemisphere carbon monoxide correction into the 15°S-50°S band instead of including part of it in the >50°S band is that Tans *et al.* use a fixed Southern Ocean efflux of 0.5 Gt C yr^{-1} in all their model scenarios. The skin-temperature correction is applied to the two latitude bands in which the Tans *et al.* estimate uses the air-sea exchange estimates obtained from measurements.

† The anthropogenic CO₂ uptake estimate is taken from the simulation of ref. 4 for the same time span (1972-89) as the data set of Tans *et al.*².

associated with carbonate weathering, silicate weathering, and organic carbon production and remineralization (Fig. 1). Because these fluxes are difficult to measure, they are usually approximated using steady-state assumptions. Such assumptions are questionable. For example, Wollast and Mackenzie¹³ have suggested that anthropogenic effects may enhance CO₂ release into the ocean by as much as $\sim 0.6 \text{ Gt C yr}^{-1}$. But because non-steady-state CO₂ will be buffered by reaction with the very large pool of dissolved inorganic carbon in the ocean, the actual flux of non-steady-state CO₂ from the ocean to the atmosphere will be only $\sim 17\%$ of that produced^{4,14}. Even the pronounced effect proposed by Wollast and Mackenzie should only increase CO₂ evasion by $\sim 0.1 \text{ Gt C yr}^{-1}$. Thus we believe that steady-state-based estimates are a reasonable representation of the present-day geochemical CO₂ budget.

For the combined carbonate and silicate geochemical cycles (Fig. 1a, b), the magnitude of the flux of CO₂ out of the ocean can be estimated as the difference between carbon entering the ocean as dissolved inorganic carbon (primarily HCO₃⁻) in rivers, and the loss of carbon to the sediments as CaCO₃ (ref. 15). Table 2a summarizes recent estimates for these two quantities. To complete the geochemical steady state, a separate source of CO₂ is necessary to balance the CO₂ consumed during silicate weathering and subsequently buried in carbonate sediments (Fig. 1b). This source of CO₂ is thought to be the decarbonation of carbonate minerals during metamorphism and volcanism. The flux of CO₂ produced by decarbonation can enter the atmosphere either directly or through the ocean. Because the ocean component of the decarbonation flux is very uncertain¹⁶, the total decarbonation flux is treated as an uncertainty in Table 2a. Given the uncertainties, we estimate that the carbonate and silicate geochemical cycles require an ocean-to-air CO₂ flux of $\sim 0.2-0.3 \text{ Gt C yr}^{-1}$.

Like the carbonate and silicate cycles, the geochemical organic carbon cycle can be approximated as a steady state for the purpose of deriving preliminary estimates of the implied CO₂ flux. Perhaps the simplest steady-state approach is to assume that there is a flux of CO₂ out of the ocean equal to the organic carbon flux into the ocean by rivers, minus the burial of organic

carbon in marine sediments¹⁷. From river flux estimates (Table 2b) we conclude that the most likely total organic carbon load in rivers globally is $\sim 0.3-0.5 \text{ Gt C yr}^{-1}$. The burial of organic carbon in sediments has been estimated^{17,18} at $\sim 0.1 \text{ Gt C yr}^{-1}$, giving an implied CO₂ flux of $0.2-0.4 \text{ Gt C yr}^{-1}$ that must be transferred from the oceans to the land by way of the atmosphere (Table 2c).

A considerable uncertainty affecting our hypothesis is the extent to which estuarine and continental shelf processes alter carbon transfer from rivers to the open ocean. The principal sink for riverine inorganic carbon is carbonate sedimentation, which occurs almost exclusively in waters removed from fresh water inputs. The distribution of dissolved organic carbon seems to be conservative in many estuaries¹⁹. The fate of particulate organic carbon in estuaries is more complex. Although some riverine organic carbon may not be transported through estuaries, additional terrestrial organic carbon may reach the oceans by transport in marine aerosols²⁰. The average air-sea CO₂ difference that would be required in shelf regions (7.5% of the ocean area) to release the entire geochemical CO₂ flux is $\sim 20-74 \text{ p.p.m.}$, assuming the range of global average gas-exchange coefficients given above. Because the equilibration time of ocean surface water with respect to CO₂ gas exchange is about one year²¹, it seems unlikely to us that the shelf regions could maintain such large global average air-sea differences without significantly affecting the open ocean. More data from coastal regions are needed, but those that are available suggest that CO₂ exchange in shelf regions is related primarily to large-scale oceanic features (such as upwelling zones) rather than local river fluxes.

We therefore conclude that the ocean CO₂ budget must be offset by $\sim 0.4-0.7 \text{ Gt C}$ to account for the steady-state geochemical fluxes (Table 1c). When combined with adjustments for the skin-temperature and carbon monoxide corrections, these fluxes bring the CO₂ budget of Tans *et al.*² to within a range that overlaps that of the revised IPCC budget¹, without requiring that the ocean models be incorrect, or that the air-sea CO₂ measurements be biased. We therefore believe that the summary given in Table 1c supports the overall conclusion that there is essentially no conflict between the estimates of uptake from ocean models and the observationally based estimates of Tans *et al.*².

It will not be easy to constrain the estimates of river carbon transport further, or to determine the latitudinal distribution of the associated CO₂ fluxes. Limited oceanic uptake of CO₂ in the Southern Hemisphere implies that a large fraction of the geochemical CO₂ efflux must effectively be leaving the ocean there (Table 3). Such an efflux may be maintained by interhemispheric southward flow of North Atlantic Deep Water²². Additional observations can be done to constrain the skin-temperature correction as well as the poorly known contribution of estuaries and shelf regions to the oceanic carbon budget. The results of Keeling *et al.*⁶ suggest that the distribution of ¹³C in the atmosphere is not consistent with the large terrestrial vegetation sink postulated by Tans *et al.*². The geochemical flux and other corrections we postulate similarly reduce the inferred terrestrial sink for anthropogenic CO₂ to a magnitude consistent with the models of ocean CO₂ uptake. Additional terrestrial carbon uptake is required, however, to supply the geochemical river fluxes (Table 1c). Future analysis of the atmospheric ¹³C distribution may provide important further constraints. □

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Seismological evidence for metastable olivine inside a subducting slab

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THE nature and location of the olivine–spinel phase transition inside subducting slabs differ greatly from the situation in the surrounding mantle, due to the different temperature distribution inside the slabs. Two models have been proposed for this phase transition: one in which the location of the phase boundary between olivine and modified (β -phase) spinel is determined by equilibrium thermodynamics¹, and the other including a metastable olivine phase which persists to a depth of ~550 km (ref. 2). The location of the olivine–spinel transition in the slab may be relevant to the generation of deep earthquakes^{3–5}, and to the buoyancy forces driving subduction⁶. Here we use travel-time residuals from deep earthquakes recorded by the dense seismograph network in Japan to investigate the configuration of the olivine–spinel phase boundary inside the subducting Pacific plate. Theoretical travel-time residuals for the equilibrium model do not fit the observed residuals, whereas those for the metastable model do, implying the presence of metastable olivine inside the subducting slab.

The seismic discontinuity near 400 km depth (now generally referenced to as the '410-km discontinuity') is generally interpreted as the result of the phase change from olivine to β -

spinel^{7,8}. Thermodynamic data for the dependence of this phase change on pressure and temperature predict that the equilibrium phase boundary inside a cold downgoing slab will be distorted upwards because of the positive Clapeyron slope of the phase change^{1,9}. On the other hand, under non-equilibrium conditions, metastable olivine may exist inside a cold slab at depths of 400 km or greater^{2,10}.

The question of whether metastable olivine exists in downgoing slabs has important geophysical implications. Recent mineralogical experiments suggest that the occurrence of deep earthquakes may be related to a phase change of metastable olivine^{3–5} to a high-pressure phase. The existence of metastable olivine would also affect the driving force of the downgoing slab, because the large density difference between the metastable olivine and the β or γ phases of the surrounding mantle would influence the buoyancy force acting on the slab⁶.

Here we analyse seismic travel-time data to determine whether metastable olivine exists in a downgoing slab. This has previously been attempted for the Tonga and Izu–Bonin slabs^{11,12}, but large errors in locating the hypocentre, due to sparse data, precluded a definitive conclusion.

We search for metastable olivine in the Pacific slab beneath southwestern Japan using travel-time data from deep events which took place beneath this region. We calculate theoretical travel-time residuals for two models. In the first⁹ (called the equilibrium model hereafter) the olivine– β -phase boundary in the slab follows equilibrium thermodynamics; in the second^{2,10} (called the metastable model hereafter) olivine persists metastably in the downgoing slab to a depth of 550 km. The equilibrium model is based on work by Schubert *et al.*⁹ and the metastable model is based on work by Liu¹⁰. The dense seismic network in southwestern Japan minimizes the location errors of deep earthquakes beneath this region and enables us to investigate the detailed velocity structure in the slab.

We analyse P-wave arrival-time data from 29 events with depths ranging from 300 to 500 km beneath southwestern Japan and with magnitudes larger than 4.0. We use 63 seismograph stations from the seismic networks of the Earthquake Research Institute of the University of Tokyo and the National Research Institute for Earth Science and Disaster Prevention. These stations were chosen because they are located as close as possible to the up-dip direction of the slab (Fig. 1). The reading error

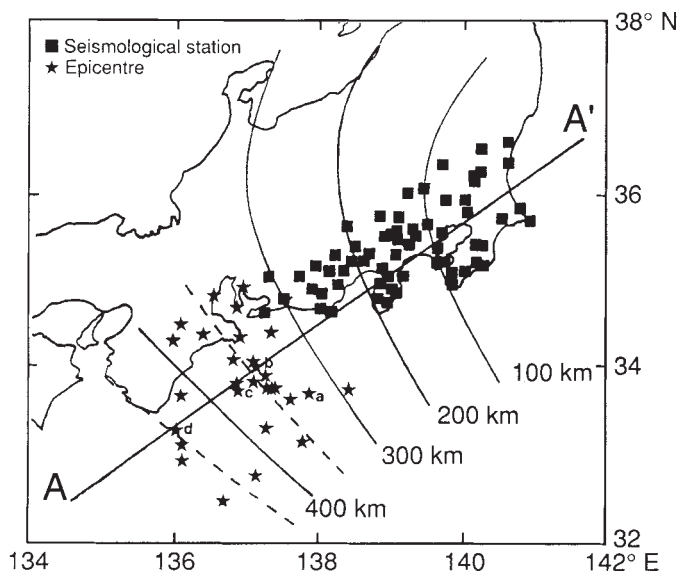


FIG. 1 Deep earthquake epicentres (stars) and seismic stations (squares). Iso-depth lines (in km) of the deep seismic zone are shown by solid contours. The points labelled a, b, c and d are the master events used for relocation of the hypocentres. Ray tracing along profile A–A' is shown in Fig. 2.

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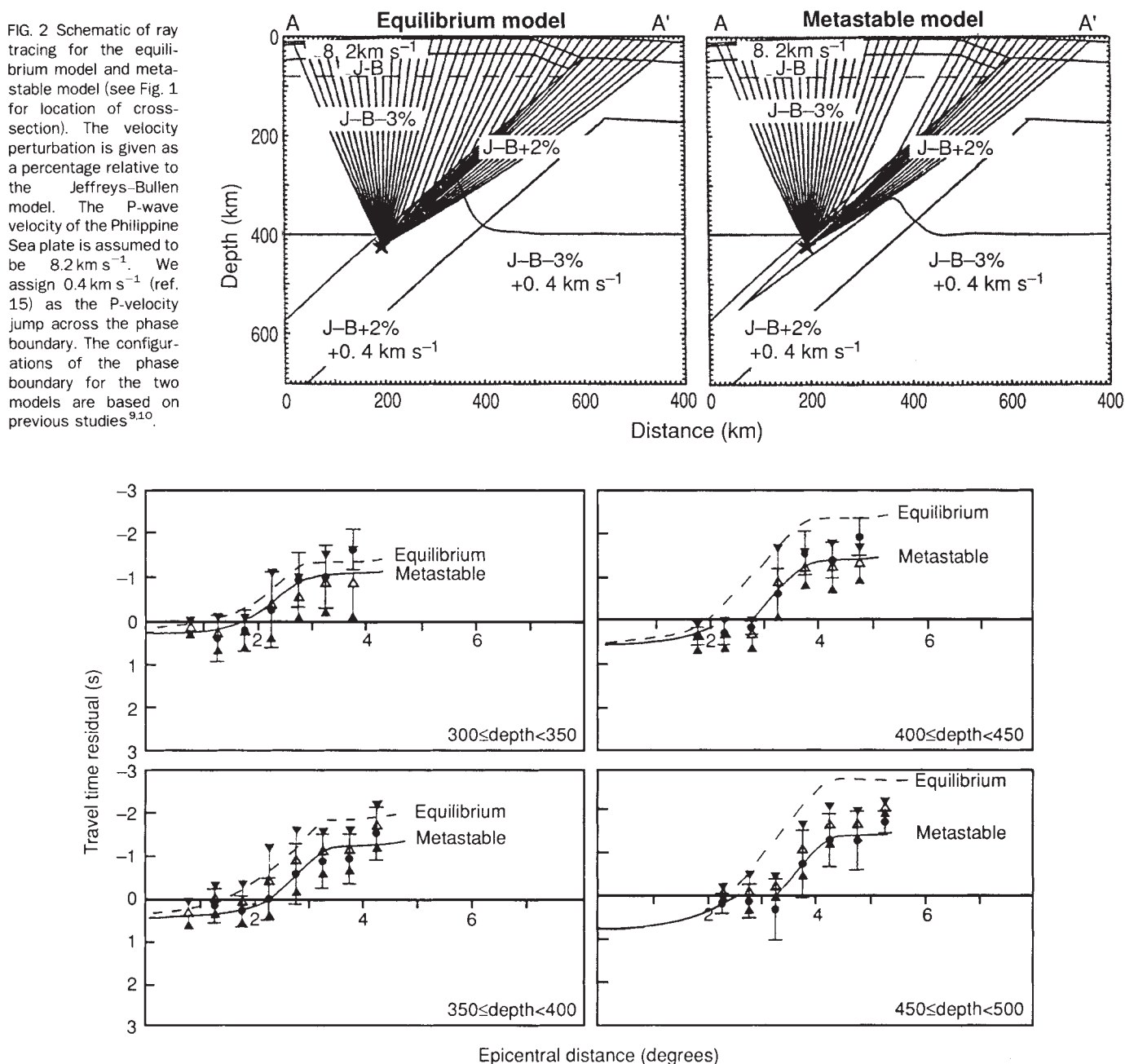
of the arrivals, which are taken from bulletins, is less than 0.3 s.

We relocate the hypocentres determined by the International Seismological Centre (ISC) as follows. We select four large earthquakes ($m_b > 4.9$) as 'master events', because larger earthquakes can be more accurately located. The master events are relocated using P and S arrival-time data. We adopt the ISC pP–P depths as the focal depths for these events. We relocate the other earthquakes by comparing their P and S arrival times with those of the master events. This procedure improves the accuracy of absolute location of all the earthquakes.

When there are only a few seismograph stations above the deep events, the ISC hypocentres may be biased because of the high-velocity slab. But because of the high density of seismograph stations, the ISC hypocentres in the region chosen are

very close to hypocentres which were relocated taking into account the high-velocity Pacific slab and low-velocity mantle wedge¹³. Here we relocate the hypocentres to minimize the effect of the high-velocity slab. We use both P and S arrivals in the relocation. Travel-time data at seismic stations in the northeast region of Japan are particularly affected by the subducting high-velocity slab¹³. To eliminate the effect of these stations, we relocated the hypocentres without using data from Japanese stations located east of 138° E. Below, we present residuals for both the ISC and relocated hypocentres.

We calculate theoretical P-residuals for the equilibrium model and metastable model using two-dimensional ray-tracing, and compare the theoretical residuals to the observed P-residuals. The residuals are calculated with respect to the Jeffreys–Bullen model; station corrections obtained previously¹⁴ are used. Figure



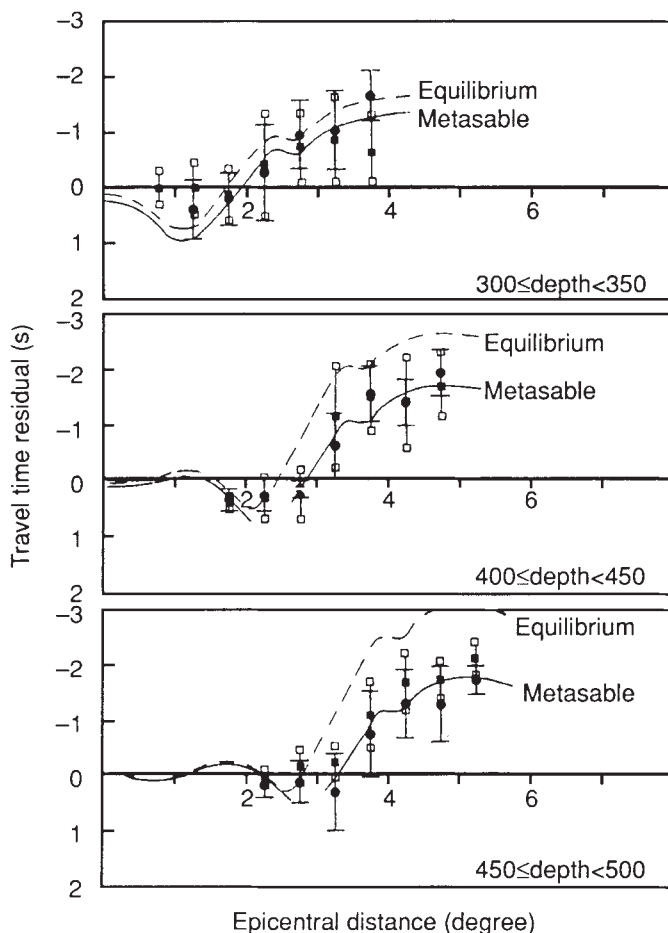


FIG. 4 Observed (solid circles, error bars, solid squares and open squares) and theoretical travel-time residuals. Solid circles and error bars denote averaged residuals and one standard deviation for ISC hypocentres. Solid and open squares denote, respectively, averaged residuals and one standard deviation for the relocated hypocentres. A modified model with a heterogeneous slab¹⁷ and a mantle wedge¹⁹ is used. Theoretical residuals for the equilibrium model and the metastable model are shown by dashed and solid lines, respectively.

The residuals for master event 'c' are shown in Fig. 3, but the residuals for the other three master events are substantially the same. We therefore conclude that the superior fit of the metastable model is real, and is not an artefact of mislocated hypocentres.

To assess how far this result depends on the model, we slightly modified both models by introducing lateral heterogeneity within the slab on the basis of thermal modelling¹⁷, and by introducing lateral heterogeneity in the mantle wedge on the basis of results from seismic tomography^{18,19}. The metastable model fits the observed residuals much better than the equilibrium model for all of the cases tested (Fig. 4). The relocated hypocentre data, which minimize the effect of possible errors due to the subducting high-velocity slab, also show that the metastable model fits the observed data much better than the equilibrium model.

These results may have considerable implications for the source process of deep earthquakes. Recent experiments³⁻⁵ suggest that a new type of shear instability may be responsible for deep earthquakes. Our evidence for the existence of metastable olivine in the Pacific slab at depths below 400 km is consistent with the shear instability hypothesis. The depth to which the metastable olivine wedge extends cannot, however, be resolved here, partly because the maximum depth of seismicity in the studied region is only ~500 km. To find the depth of the metastable region, analysis of amplitude and waveform data as well as travel-time data for both upgoing and downgoing P-waves is required. □

2 shows the results of ray-tracing calculations for the two velocity models. We use the P-velocity model of Suyehiro and Sacks¹⁵ as the velocity structure of the surrounding upper mantle for both models. In the surrounding upper mantle, the α - β phase boundary is located at a depth of 400 km. We assign 0.4 km s^{-1} (ref. 16) as the P-velocity jump across the phase boundary.

We divide the residual data into four groups: hypocentral depth ranges of 300–350 km, 350–400 km, 400–450 km and 450–500 km (Fig. 3). For each depth range, the theoretical residuals are calculated by ray tracing from a hypothetical source at the midpoint of each depth range, located 20 km from the assumed upper boundary of the Pacific plate. The results shown in Fig. 3 provide strong support for the existence of metastable olivine in the slab at depths below 400 km. It is important to note that the predicted variation of the residuals with increasing epicentral distance for both the equilibrium and metastable models fits the data for the hypocentres at depths from 300 to 350 km. As the events become deeper, however, the predicted residuals for the equilibrium model diverge increasingly from the observed data.

If one were to consider only the 450–500-km results, it would be necessary to consider the possibility of systematic errors due to hypocentral mislocation. That is, the equilibrium model could be correct, and the apparent misfit could arguably be attributed to location errors. But when the datasets for all four depth ranges in Fig. 3 are considered together, this possibility is effectively eliminated, as the misfit for the equilibrium model only occurs for depths greater than 400 km; if the residuals were due to a systematic error in hypocentral location, this systematic error would have to increase linearly with depth. We also calculated the residuals for the relocated hypocentres using the master event method to improve the accuracy of relative locations. As shown in Fig. 3, there is essentially no difference between the residuals for the ISC hypocentres and the relocated hypocentres.

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Olfactory cues influence female choice in two lek-breeding antelopes

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PRONOUNCED differences in mating success between males holding territories clustered on traditional mating grounds (leks) are commonly cited as evidence of female choice for male phenotypes¹⁻⁶, but female ungulates appear to prefer particular territories⁶⁻¹² even when no other individuals are on the lek^{11,12}. Female choice of territories may be influenced by spatial features^{7-10,12}, but observations suggest that females may also be attracted to successful territories by olfactory cues in the soil¹³. Here we report that transferring the topsoil between successful and unsuccessful territories on leks of two reduncine antelope species caused the numbers of females and matings on the unsuccessful territories to increase tenfold. Females were probably attracted to the soil by smells that had accumulated from heavy use by other females. Because of this attraction, stochastic process may play an important part in generating the variance in mating success between territory holders on leks.

Two reduncine antelopes, Uganda kob (*Kobus kob thomasi*) and Kafue lechwe (*Kobus lechwe kafuensis*), mate on leks^{14,15}. In both species, oestrus females are attracted to leks¹⁶ and congregate on a few territories where the majority of matings occur^{7-9,12,16}. Although the males defending the most successful territories are replaced every one or two days, the territories remain successful for months^{7,12,16}. The mating rates of different males holding the same territory are thus highly correlated¹², and when a male kob changes territories, his success on the new territory is predicted by the success of the previous occupant (Spearman's $r_s = 0.56$, $n = 30$, $P(\text{two-tailed}) = 0.0027$), not by his own previous success ($r_s = -0.088$, $P = 0.63$). One explanation is that females choose territories on the basis of spatial characteristics, such as distance to the centre of the lek⁷⁻⁹, to lek entry paths¹⁰ or to cover that can hide predators¹², but this would not explain the large differences in mating success between adjacent territories¹². An additional explanation is that females are attracted to heavily used territories by olfactory cues¹³. Oestrus females coming onto leks smell the ground repeatedly ($1.16 \text{ sniffs min}^{-1}$; $n = 35$ lechwe), and successful territories, which smell strongly to humans, are dotted with yellow patches where females have urinated for soliciting males^{7,13}.

To test whether females are attracted to territories by olfactory cues, we independently carried out experiments on kob in Queen Elizabeth National Park, Uganda, and on lechwe in Lochinvar National Park, Zambia, during April–July 1991. J.C.D. and two assistants observed a kob lek of about 17 males for 35 days, each day identifying all males, mapping their locations, recording all matings, and scanning female locations every half-hour. After seven days, each of the two most successful territories was paired with a neighbouring unsuccessful territory and experimental and control pairs chosen at random. About 250 kg of soil and dead grass were removed from each of the four territories, the earth exchanged between the two experimental territories, and the original earth replaced on the two controls. This procedure was repeated on four new territories each week for four weeks. R.J.C.N. and three assistants collected similar data on a lechwe lek of about 80 males, identifying a quarter of the males. After 10 days of observation, 300 kg of topsoil was transferred from each of the 11 most successful lechwe territories to 11 unsuccessful ones, and black polythene sheets (9 m^2) were placed on the successful territories for one day to force females

to choose between the other territories on the lek. Observations were carried out for five days after the removal of plastic sheets. The plastic sheets were used in lechwe because it was expected that individual females would return to the same territories immediately after the manipulation, and that some scent would remain on these territories despite the treatment.

In both species, the number of matings on the unsuccessful territories to which earth had been moved increased more than 10-fold (Fig. 1), whereas the numbers on control and unmanipulated territories remained unchanged. In kob, 39 matings occurred on the four experimental territories and 0 on the four controls (Table 1), whereas in lechwe, 9 occurred on the 11 experimental territories and 2 on the 57 other previously unsuccessful territories. If matings had been randomly distributed between experimental and control or other territories, the probability of obtaining results this extreme would have been very small (randomization tests: kob, $P < 0.0001$; lechwe, $P < 0.0001$). In lechwe, moreover, the increase in mating success on territories to which earth had been moved was significantly correlated with the success of territories from which the earth had been taken (Fig. 2). The mean number of females per territory per day also increased on experimental territories in both species (Mann-Whitney U tests: kob, $n_1 = 28$, $n_2 = 28$, $P(\text{one-tailed}) = 0.014$; lechwe, $n_1 = 110$, $n_2 = 55$, $P < 0.0001$), but not on controls or unmanipulated territories (kob, $n_1 = 28$, $n_2 = 28$, $P = 0.37$; lechwe, $n_1 = 570$, $n_2 = 285$, $P = 0.059$).

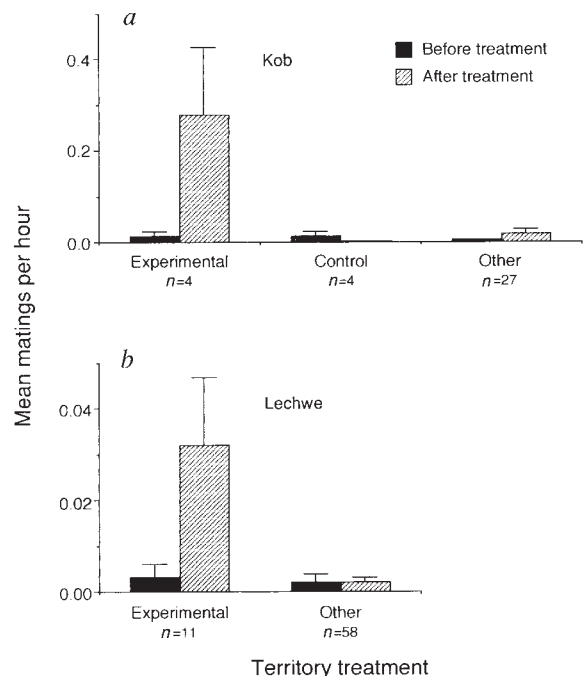


FIG. 1 Mating rates on experimental, control and other territories on kob and lechwe leks before and after transferring earth to the experimental territories from successful territories (means $\pm$ s.e. of territories). The probability of obtaining results this extreme if matings had been randomly distributed between experimental and control or other territories after manipulation would be very small (see text). If females copy each others' choices visually²¹⁻²⁵, however, matings would not be independent, so we also tested the significance of the change in mating rates on experimental territories compared with the change in other territories, taking territories as sampling units. a, In kob, there was no difference between changes in mating rates on controls and on other previously unsuccessful territories (Mann-Whitney U test: $n_1 = 4$, $n_2 = 27$, $P(\text{two-tailed}) = 0.13$), so these categories were combined. Because it was extremely rare for the mating success of an unmanipulated territory to increase substantially over two weeks, the increase on experimental territories was highly significant compared with changes on controls and others (Mann-Whitney, $n_1 = 4$, $n_2 = 31$, $P(\text{one-tailed}) = 0.0008$). b, In lechwe, the increase on experimental territories was also significant compared with changes on other previously unsuccessful territories (Mann-Whitney: $n_1 = 11$, $n_2 = 57$, $P = 0.014$).

Although the moved earth seemed to attract females directly, an alternative explanation of these results is that the treatment attracted more competitive males whom females preferred. In kob, where all males were identified, the data on individual success does not support this alternative. Although more males held experimental territories after manipulation than before, indicating heightened competition, matings per male also increased (Table 1). Furthermore, three of the four individual males who held experimental territories both before and afterwards achieved more matings afterwards, and there was no apparent difference between the success of these males and those that replaced them (Table 1). Mating success tended to decrease on the successful territories from which earth had been taken, but several factors may have been involved. In kob, mating rates tended to drop (from 0.51 to 0.27 per h; Wilcoxon signed-ranks test: $n = 4$ territories, $P(\text{one-tailed}) = 0.14$), but rates also tended to drop on control territories (from 0.56 to 0.38; $n = 4$, $P = 0.28$), so part of the decrease may have resulted from disturbance or from regression towards the mean. In lechwe, where successful territories were covered for one day by plastic sheets, their success after the sheets were removed was significantly lower than before manipulation (mean before = 0.031, mean after = 0.0036; $n = 11$, $P = 0.045$).

Although the experiment showed that females can be attracted to territories by olfactory cues, it did not demonstrate whether these cues accumulate from use by males or by other females. One possibility is that females choose between territories based on the quality of scent deposited by individual males. This would not explain, however, why one territory may remain the most successful on a kob lek for several months in spite of being held by over 20 males per month¹², nor why males with superior scents should compete to hold this territory for one or two days rather than taking over an adjacent territory which could be held for much longer¹⁶. Scent marking by males, although present in another lekking antelope¹⁷, is also conspicuously absent in kob¹⁸. A second possibility is that females choose territories not by the quality of smell but by the concentration of one or more substances in the soil. These might accumulate from use by either or both sexes, but large differences between successful and unsuccessful territories would be more likely to stem from differences in use by females: while territories are each occupied by a single male, the mean number of females is much higher on successful than unsuccessful territories (35 times higher in kob: Mann-Whitney, $n_1 = 8$ territories on which > 1 matings occurred, $n_2 = 8$ territories on which ≤ 1 mating occurred, $P(\text{two-tailed}) = 0.0016$; 113 times higher in lechwe, $n_1 = 11$, $n_2 = 57$, $P = 0.0002$), and oestrus females urinate frequently for soliciting males⁷.

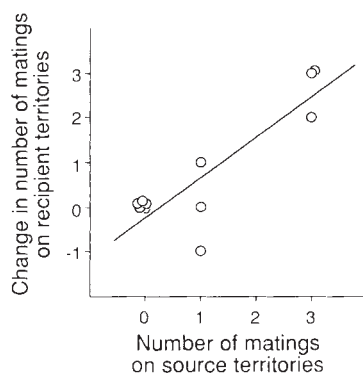


FIG. 2 The relationship between change in mating success of lechwe territories before and after receiving earth and the previous mating success of territories from which the earth had been taken (Spearman's $r_s = 0.70$, $n = 11$, $P(\text{two-tailed}) = 0.026$). Matings were recorded during 35.5 h before and 25.5 h after manipulation.

TABLE 1 Numbers of matings attained by individual male kob holding experimental and control territories

	Experimental		Control	
	Before	After	Before	After
Run I	0*	4* 2 1 1	0	—
Run II	1*	3* 1	0 1	0 0
Run III	0 0*	3* 2 8 9 3	1	0
Run IV	1*	1* 0 1 0	0 0*	0 0*

Matings were observed for 35 h before and 35 h after each experiment. Asterisked numerals indicate males who held the same territory before and after its manipulation. Although more males held experimental territories after earth moving than before, the number of matings per male also increased (Mann-Whitney U test, $n_1 = 5$, $n_2 = 15$, $P(\text{one-tailed}) = 0.022$). Three of the four individuals who held experimental territories both before and afterwards achieved more matings afterwards, and there was no apparent difference between the success of these males and those that replaced them (Mann-Whitney: $n_1 = 4$, $n_2 = 11$, $P(\text{two-tailed}) = 0.28$).

If females are attracted by the smell of female urine, some component of female urine must accumulate in the soil of successful territories, enabling them to be distinguished from unsuccessful ones. We tested this by collecting paired soil samples from the most successful territory and from an adjacent unsuccessful one on seven different kob leks and assaying the levels of oestrone 3-sulphate^{19,20}, a derivative of oestrogen found in the urine of oestrus female ungulates¹⁹. Concentrations averaged 30.9 ng g^{-1} dry soil on successful territories, 19.6 ng g^{-1} on unsuccessful ones, and on all seven leks the levels were higher on the successful than the unsuccessful territory (Wilcoxon, $n = 7$, $p(\text{two-tailed}) = 0.016$). But application of synthetic oestrone (sodium salt of 1,3,5(10)-estratrien-3-ol-17-one

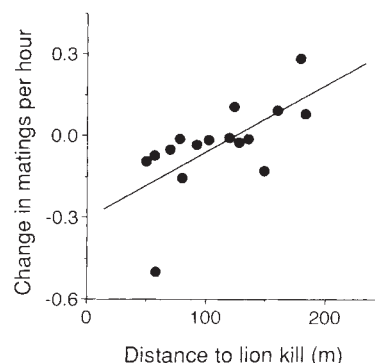


FIG. 3 The association between change in mating success of territories on the kob lek before and after a lion kill and distance from each territory to the kill, showing the 16 non-experimental territories on which at least one mating occurred (Spearman's $r_s = 0.65$, $n = 16$, $P(\text{two-tailed}) = 0.011$). The relationship is similar for all 25 non-experimental territories occupied by a male before and after the kill ($r_s = 0.62$, $n = 25$, $P = 0.0026$). Mating rates (total matings divided by total hours occupied by a male) were recorded during 140 h over 28 days before the kill and 35 h over 7 days afterwards.

sulphate and 3-hydroxy-1,3,5(10)-estratrien-17-one 3-sulphate) to territories on another lek did not attract females; some other constituent of female urine may be involved.

One consequence of female attraction to heavily used territories is that any factor that generates irregularities in female distribution on leks may have an exaggerated effect on differences between the matings success of males. In kob, one such factor is predation. Leks located in densely thicketed areas show positive associations between the success of male territories and their distance to thickets¹², and experimental manipulation of grass height has shown that females also choose between different leks partly on the basis of visibility¹². During data collection for the earth-moving experiment, a pride of lions

killed a male kob on the edge of the lek, and this event was associated with a persistent decline in the mating success of nearby territories and rise in the success of distant ones (Fig. 3). The influence of the lion kill on male success appears to have been prolonged by females' attraction to sites used by previous females. In other species, or even on other kob leks, female distribution may be affected by other territory characteristics^{10,16} or by traits of particular males^{3,4,16}. But if chance events frequently alter female distribution, then attraction to heavily used territories, like visual copying by females of other females' mate choices²¹⁻²⁵, may increase the variance in male mating success^{26,27} without increasing the strength of sexual selection²⁵. □

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Amphotericin B treatment dissociates *in vivo* replication of the scrapie agent from PrP accumulation

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SCRAPIE and related animal and human disorders are neurodegenerative diseases¹ characterized by the formation of a modified, partly proteinase-resistant protein (PrP) of the host^{2,3}, which tends to aggregate as amyloid fibrils⁴ and accumulate in the brain of infected individuals⁵. There is a general consensus that the pathological form of PrP (PrP^{Sc}) is essential for the clinical appearance of the disease⁶, but whether it is part of the scrapie agent⁷ or a by-product of viral infection^{8,9} is still controversial. Here we report that treatment of scrapie-infected hamsters with amphotericin B delays the accumulation in the brain of the proteinase-resistant portion of PrP^{Sc} by about 30 days without affecting scrapie replication. The consequence is that hamsters treated with amphotericin B developed clinical signs of disease later than infected controls. We argue that the proteinase-resistant portion of PrP^{Sc} is necessary for the development of the disease but that it is unlikely to be essential for scrapie replication.

We have previously reported that treatment with the polyene antibiotic amphotericin B prolongs the incubation period of hamsters infected intracerebrally (i.c.) with strain 263K of

scrapie¹⁰. The beneficial effect of the antibiotic correlates with its concentration in the brain¹¹, which depends on the amount of the dose injected and on the duration of the treatment. But in spite of daily maximal administration of non-toxic amounts of amphotericin B, hamsters develop scrapie, albeit later than non-treated scrapie-infected hamsters¹⁰. Considering possible mechanisms of action, we proposed¹² (1) that amphotericin B might modify a hypothetical receptor molecule for the scrapie agent on target cells of the central nervous system (CNS), or (2) that it delays replication of the scrapie agent, or (3) that it might interfere with the formation and accumulation of the pathological form of PrP in the brain of infected animals. This last idea presumes that scrapie replication is not affected by amphotericin B unless PrP is a component of the scrapie itself. These three possibilities may not be mutually exclusive if PrP is a scrapie agent receptor and PrP agent-binding promotes the accumulation of PrP^{Sc} and of infectivity.

To test the first hypothesis, we treated two groups of hamsters from the same colony with two different hamster-adapted strains of scrapie (263K and 139H) (Table 1). Amphotericin B significantly ($P < 0.001$) delayed the appearance of clinical signs of disease in hamsters infected with 263K strain of scrapie, as previously reported^{10,12}, but it did not have any beneficial effect in hamsters infected with 139H (Table 1). Moreover, there was no effect in Swiss mice infected i.c. with strain 139A of scrapie, which is the parental and equivalent strain of 139H in hamster¹³. Unfortunately, it is not possible to test the effectiveness of the antibiotic in mice infected with scrapie strain 263K because this strain is poorly pathogenic in mice¹³. Thus, the anti-scrapie effect of amphotericin B is strain-specific and probably does not depend on the modification of a cellular component, unless the antibiotic modifies only the receptor specific for strain 263K. This assumes that there are different receptors or different sites of the same receptor for different strains of scrapie.

The second and third hypotheses were tested simultaneously. The proteinase-resistant portion of PrP^{Sc}, PrP27-30, and scrapie infectivity were measured in the same fraction of brains (see

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Fig. 1 legend) removed from non-treated and amphotericin B-treated hamsters at different intervals after i.c. injection with scrapie. This procedure was chosen because PrP27-30 and scrapie infectivity copurify in the same fraction¹⁴. Scrapie replication in the brain of both non-treated and antibiotic-treated hamsters started soon after the i.c. injection of the inocula and proceeded in an exponential way up to about 8 log LD₅₀/brain equivalent (Fig. 1a). Afterwards scrapie replication remains at an almost constant level until the death of the animal. In both groups the concentration of the scrapie agent reached a plateau before the appearance of clinical signs of disease. The effect of the antibiotic on scrapie replication in the brain was negligible. The difference between the estimated infectivity titres in brains of non-treated and treated animals killed at the same time after inoculation was always less than 0.5 logs, except for the 32-day samples, which were 0.7 logs.

By contrast, treatment with amphotericin B delayed by 30 days the appearance of PrP27-30 in the brain compared with non-treated animals (Figs 1b and 2). PrP27-30 was detectable in the brain of non-treated hamsters from the twenty-second day after i.c. inoculation and reached its maximum concentration in the next 40 days (Fig. 1b). In treated animals, however, PrP27-30 was measurable only after 52 days, when the estimated infectivity titre was 6.6 log LD₅₀/0.1 brain equivalent. The corresponding point in non-treated hamsters had a scrapie titre of only 0.3 log higher than in amphotericin B-treated animals (Fig. 1a) but with 100-fold more PrP27-30 (Fig. 1b).

In order to have a better estimate of the effect of amphotericin B treatment on scrapie replication and PrP accumulation we preferred to perform statistical analysis on the curves instead of comparing the single experimental points with paired *t*-tests¹⁵. This was accomplished in two steps. First the two sets of experimental points were separately fitted and then the data were combined and fitted simultaneously. The *F* test was used to determine whether the two sets of data are well fitted by one curve. The *F* test indicated that the rate of scrapie replication did not differ between non-treated and amphotericin B-treated animals (Fig. 1a; *P* > 0.1), whereas the rate of increase of

PrP27-30 in the brain of treated animals was significantly (Fig. 1b; *P* < 0.01) dissimilar from that of non-treated hamsters.

The critical time points of this experiment were repeated and fully confirmed the previous results (Table 2). Moreover, the sample taken 36 days after infection was also end-point titrated¹⁶ (Table 2) to prove the accuracy of the measurement of infectivity by the incubation period method¹⁷. As amphotericin B might only be inhibiting the protease-resistant form of PrP^{Sc} (PrP27-30), we purified PrP from brains of non-treated and treated

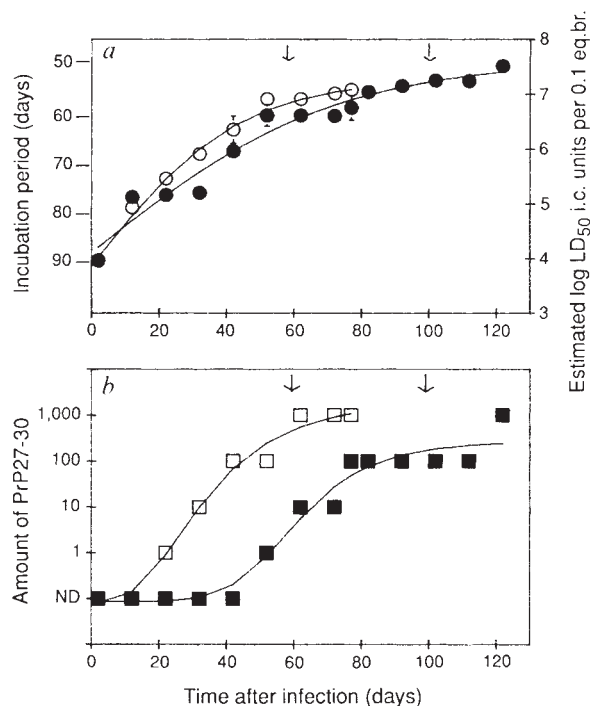


FIG. 1 Effect of amphotericin B (AmB) treatment on scrapie replication and PrP27-30 accumulation in purified brain preparations of hamsters infected i.c. with the 263K strain of scrapie. *a*, Replication of 263K in non-treated (open circles) and AmB-treated (filled circles) hamsters. Nonlinear regressions of the data using an asymptotic regression curve suitable for describing the growth curve of the scrapie agent²⁵. *b*, Accumulation of PrP27-30 in non-treated (open squares) and AmB-treated (filled squares) hamsters. The amount of PrP27-30 is expressed as in Table 2. Nonlinear regressions of the data to a symmetrical sigmoidal logistic curve used for the analysis of physiological dose-response curves²⁶. The chi-square goodness of fit and the runs test revealed that the curves did not deviate from the experimental points and that the equations were therefore appropriate for the data. Arrows in *a* and *b* indicate the appearance of scrapie disease in donor hamsters.

METHODS. PrP27-30 was purified from frozen brains and spleens of 263K-infected hamsters according to published procedures^{27,28}. The final pellets were suspended in 150 μ l (50 μ l per brain) of 10 mM Tris-HCl, pH 7.4, 0.1% SB 3-14 and used both for the measurement of PrP27-30 and for the determination of infectivity titre. In order to avoid cross-contamination, each step of the purification of PrP27-30 was done in sequential order from brains of animals killed 2 days after inoculation onwards and from AmB-treated to non-treated hamsters. Whenever possible, disposable material was used. The sonicator tip was wiped dry after each sonication, rinsed with absolute alcohol, flamed, rinsed with sterile PBS, and dried before the next sonication. An aliquot of each preparation of PrP27-30 (50 μ l per brain equivalent) purified from non-treated and AmB-treated animals was diluted 1:10, sonicated for 3 $\times$ 15 s, and aliquots of 0.05 ml (0.1 brain equivalent) were injected i.c. into 6 recipient hamsters. Incubation periods were measured and infectivity titres estimated by applying these values to a dose-incubation curve¹². Another aliquot (1 brain equivalent) of the same purified fraction was used to measure PrP27-30 by western blotting (Fig. 2). If PrP27-30 was detected, then the sample was diluted 10-fold up to 1:10,000 (corresponding to 0.0001 brain equivalent) until the minimal amount of PrP was observed (see Fig. 2 as an example). Equations were solved by nonlinear regression analysis using a computerized iterative program (EUREKA: The Solver; Borland International, Scott Valley, California).

TABLE 1 Effect of AmB treatment in animals infected with different strains of scrapie

Animal	Strain of scrapie	Dose of AmB treatment (mg/kg)	Mean incubation period (days) (mean $\pm$ s.d.)	Difference from control (days)
Hamster	263K	0	56.6 $\pm$ 3.7 (17)	—
Hamster	263K	1	98.6 $\pm$ 2.7 (9)	42.0
Hamster	139H	0	138.1 $\pm$ 3.3 (20)	—
Hamster	139H	1	138.2 $\pm$ 1.0 (13)	0.1
Mouse	139A	0	131.4 $\pm$ 3.9 (17)	—
Mouse	139A	3	131.9 $\pm$ 7.3 (12)	0.5

Outbred weanling golden Syrian hamsters and Swiss white mice were injected i.c. with 10% (w/v) suspension of scrapie-infected brains from clinically affected animals. Hamsters received 0.05 ml of either 263K (ref. 22) or 139H (ref. 23) strains of scrapie, and mice received 0.03 ml of 139A strain of scrapie²⁴. Hamsters and mice were daily scored for the presence of clinical signs¹⁰. Amphotericin B (AmB) (Fungizone; Bristol-Myers Squibb, Rome) was diluted with 5% glucose, and 0.5 ml was injected intraperitoneally (i.p.) 6 d/w. Controls were scrapie-inoculated animals that received 0.5 ml sodium deoxycholate solution in phosphate buffer at the same concentration used to dissolve the antibiotic. The treatment for 263K-injected hamsters was always started the day after the inoculation and was given until the animals were ill. Treatment of 139H- and 139A-infected animals was started the same day of inoculation and was maintained for 130 and 90 days, respectively. Mice were treated with a dose of AmB per kg body weight that was three times higher than that given to hamsters. This dose was chosen so that both animals received the same amount of AmB regardless of body weights. Clinical symptoms of AmB-treated animals were the same as those of non-treated hamsters.

TABLE 2 Scrapie replication and PrP27–30 accumulation in the brain of non-treated and AmB-treated hamsters after i.c. infection

Time after i.c. inoculation (days)	AmB-treated				Non-treated			
	Mean incubation period (days) (mean ± s.d.)	(n)	Estimated titre*	Amount of PrP†	Mean incubation period (days) (mean ± s.d.)	(n)	Estimated titre*	Amount of PrP†
36	59.0 ± 2.1	(6)	6.75 6.64‡	ND	57.8 ± 1.2	(6)	6.87 6.84‡	100
36	59.2 ± 2.4	(6)	6.74	ND	57.5 ± 1.0	(6)	6.90	100
42	58.0 ± 0.9	(6)	6.85	1	55.7 ± 1.4	(6)	7.06	100
42	57.5 ± 1.5	(6)	6.90	1	54.7 ± 1.0	(6)	7.16	100
47	55.2 ± 0.8	(6)	7.11	1	52.2 ± 1.5	(6)	7.39	100

* Estimated titre (mean ± s.e.) log₁₀ LD₅₀/0.1 brain equivalent in recipient hamsters based on a dose–incubation curve published previously¹².
† Amount of purified PrP27–30 expressed as the highest dilution of 1 brain equivalent at which PrP27–30 is still detectable; ND, undetectable in one brain equivalent.
‡ Infectivity titre based on end-point method¹⁶.

hamsters killed 40 days after i.c. scrapie inoculation using a protocol that did not include proteinase K. Samples of these purified fractions of PrP33–35 were then treated with proteinase K to distinguish between the cellular (PrP^C) and the pathological (PrP^{Sc}) isoforms. The amount of PrP33–35 in non-treated hamsters was almost 100 times as much (PrP33–35 was still detectable in 0.01 brain equivalent) as that found in treated hamsters (PrP33–35 was undetectable in 0.1 brain equivalent). PrP33–35 was mostly formed by PrP^{Sc} because it was partially resistant to proteinase K (Fig. 3).

We also removed the spleen from three non-treated and three treated animals 42 days after scrapie inoculation and purified scrapie infectivity as for the brain. We calculated the infectivity titre by measuring incubation periods in two groups of six animals injected i.c. with 0.2 spleen equivalent. The incubation periods of the recipient hamsters were 86.2 ± 2.1 (mean ± s.d.) and 86.3 ± 2.3 for non-treated and treated hamsters, respectively. This result confirms that treatment with amphotericin B does not interfere with the replication of the scrapie agent.

Our results support the hypothesis that the antiscrapie effect of amphotericin B is a result of the inhibition of the formation of the amyloid scrapie-specific protein PrP^{Sc} from the cellular precursor PrP^C (refs 12, 18). We suggest that amphotericin B works by interfering either with a strain-specific (that is, 263K) component of the infectious agent responsible for the modification of PrP^C to PrP^{Sc}, or with the 263K-specific binding site on PrP^C. This is well illustrated by its pronounced difference in effect in hamsters infected with the 263K strain and in hamsters infected with 139H (Table 1). If amphotericin B interacts with a scrapie strain-specific component, it is not apparently necessary for the replication of the infectious agent. In fact, treatment does not delay scrapie replication either in the brain or in the spleen of infected hamsters. An increasing amount of scrapie agent renders the small amount of antibiotic entering the CNS¹¹ ineffective in inhibiting the pathological modification of PrP^C. This explains the inevitable manifestation of the disease, albeit with a longer incubation period, within a limit imposed by the pharmacokinetic characteristics of the antibiotic¹⁹.

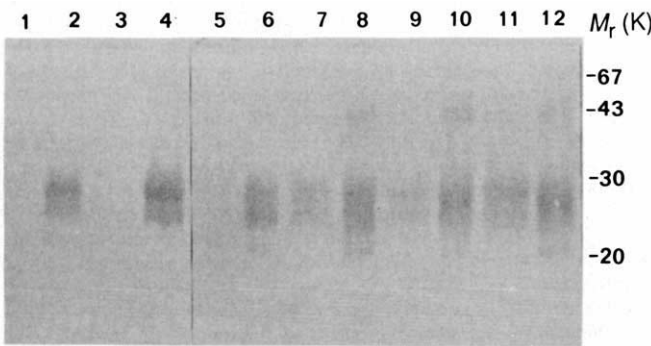


FIG. 2 Measurement by western blot of PrP27–30 in purified fraction of brain from non-treated (lanes 2 and 4, 1 brain equivalent; 6, 8, 10 and 12, 0.1 brain equivalent) and AmB-treated (lanes 1, 3, 5, 7, 9 and 11, 1 brain equivalent) hamsters after 32 (lanes 1, 2) 42 (lanes 3, 4), 52 (lanes 5, 6), 62 (lanes 7, 8), 72 (lanes 9, 10), and 77 (lanes 11, 12) days from i.c. infection. Note that PrP27–30 is clearly present in all samples of non-treated hamsters but in samples of AmB-treated animals PrP27–30 is either undetectable or its concentration is always 10–100 times lower. The most striking difference is between lane 5 (PrP27–30 barely detectable in 1 brain equivalent of AmB-treated hamsters) and lane 6 (PrP27–30 is strongly stained in 0.1 brain equivalent of nontreated hamsters).

METHODS. Purified PrP27–30 (50 µl) (1 or 0.1 brain equivalent) from non-treated and AmB-treated animals were electrophoresed on 15% polyacrylamide–SDS gels²⁹ and electrotransferred to nitrocellulose membranes³⁰. After blocking nonspecific binding with 3% gelatine, membranes were processed with rabbit polyclonal antibody³¹ (antibody P2–6, 1:2000) against hamster PrP27–30, then with a goat anti-rabbit IgG conjugated with alkaline phosphatase (BIO-RAD), and finally stained with naphthol/fast red solution.

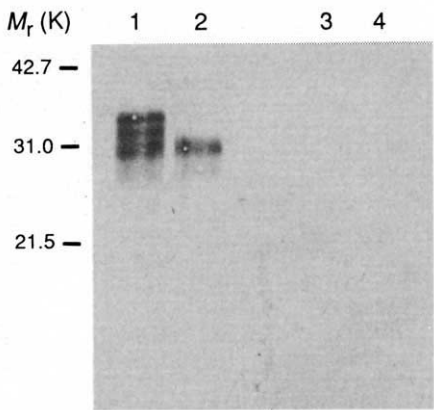


FIG. 3 Measurement by western blot of PrP33–35 and PrP27–30 in purified fractions of brains from non-treated and AmB-treated hamsters after 40 days from i.c. infection. PrP (0.1 brain equivalent) in non-treated (lanes 1, 2) and AmB-treated (lanes 3, 4) hamsters, before (PrP33–35, lanes 1, 3) and after (PrP27–30, lanes 2, 4) treatment of the purified sample with proteinase K. Note that PrP33–35 is clearly present in 0.1 brain equivalents of nontreated hamsters while in AmB-treated animals PrP33–35 is not detectable. Most of PrP33–35 found in non-treated hamsters belongs to the PrP^{Sc} isoform as it is partially protease-resistant (PrP27–30).

METHODS. PrP33–35 was purified as described for Fig. 1 except that proteinase K was omitted. After purification a sample was taken and treated with 10 µg ml^{–1} proteinase K for 2 h at 37 °C. The reaction was then stopped with 0.2 mM PMSF for 1 h at 37 °C. The proteinase K-treated and non-treated samples were electrophoresed and electrotransferred as described in Fig. 2 legend. Blots were stained as in Fig. 2.

Furthermore, our data suggest that the accumulation of PrP^{Sc} in the brain is more important than replication of the scrapie agent for the appearance of clinical signs of disease (Fig. 1a and b). This has been difficult to observe in purified fractions of brains from i.c. infected animals because PrP^{Sc} and scrapie infectivity arise at the same time⁵, being further evidence that PrP^{Sc} is a component of the scrapie agent⁷. Here we have used amphotericin B to show that PrP33–35 and its protease-resistant form (PrP27–30) are both distinguishable from infectivity, although we cannot rule out the possibility that a small amount

of the protein (not detectable) may be necessary to coat hypothesized nucleic acids, as postulated by the virino hypothesis²⁰.

According to the recently 'unified theory' of prion propagation²¹, amphotericin B might specifically interact with the 'coprion' nucleic acid of the 263K strain of scrapie, but not with that of 139H. The observed discrepancy between accumulation of PrP^{Sc} and scrapie replication in hamsters treated with amphotericin B could be explained by the ability of the 'coprion' to start its replication with a small undetectable amount of PrP^{Sc} and then to replicate independently of it. □

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Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells

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PERSISTENT changes in synaptic efficacy are thought to underlie the formation of learning and memory in the brain¹. High-frequency activation of an afferent excitatory fibre system can induce long-term potentiation^{2,3}, and conjunctive activation of two distinct excitatory synaptic inputs to the cerebellar Purkinje cells can lead to long-term depression of the synaptic activity of one of the inputs⁴. Here we report a new form of neural plasticity in which activation of an excitatory synaptic input can induce a potentiation of inhibitory synaptic signals to the same cell. In cerebellar Purkinje cells stimulation of the excitatory climbing fibre synapses is followed by a long-lasting (up to 75 min) potentiation of γ -aminobutyric acid A (GABA_A) receptor-mediated inhibitory postsynaptic currents (i.p.s.cs), a phenomenon that we term rebound potentiation. Using whole-cell patch-clamp recordings in combination with fluorometric video imaging of intracellular calcium ion concentration, we find that a climbing fibre-induced transient increase in postsynaptic calcium concentration triggers the induction of rebound potentiation. Because the response of Purkinje cells to bath-applied exogenous GABA is also potentiated after climbing fibre-stimulation with a time course similar to that of the rebound potentiation of i.p.s.cs, we conclude that the potentiation is caused by a calcium-dependent upregulation of postsynaptic GABA_A receptor function. We propose that rebound potentiation is a mechanism by which *in vivo* block of climbing fibre activity induces an increase in excitability in Purkinje cells^{5,6}. Moreover, rebound potentiation of i.p.s.cs is a

cellular mechanism which, in addition to the long-term depression of parallel fibre synaptic activity⁴, may have an important role for motor learning in the cerebellum.

Tight-seal whole-cell recordings⁷ were obtained from Purkinje cells in cerebellar slices from 12–16-day-old rats^{8,9}. With a high internal Cl[−] concentration and holding potentials of −60 or −70 mV, Purkinje cells had spontaneous synaptic inward currents which were completely blocked in the presence of bicuculline (10 μ M)⁹, identifying them as GABA_A receptor-mediated i.p.s.cs. The i.p.s.cs were recorded at a constant frequency throughout an experiment (see insets in Fig. 1), but their amplitudes tended to decay slowly under whole-cell recording conditions (see prestimulus periods in Figs 1 and 2a).

Stimulation of climbing fibres can elicit complex responses in Purkinje cells^{10,11} which under whole-cell recording conditions appear as large all-or-none excitatory synaptic currents (e.p.s.cs) accompanied by characteristic regenerative signals^{9,12}. Repetitive activation of climbing fibres (five pulses at 0.5 Hz) induced a marked potentiation of i.p.s.cs (Figs 1a, 2a and 4). Because this potentiation counteracted the spontaneous 'run-down' of i.p.s.cs, we termed it rebound potentiation. This was observed in all cells tested ($n = 6$), its duration ranging from 12 to >75 min (36 ± 22 , $n = 6$), and had a fast onset (reaching its maximum in 3–15 min after climbing fibre stimulation), followed by a slow decay phase.

Because there is evidence^{13–15} that climbing fibre stimulation induces large increases in intracellular calcium ion concentration ([Ca²⁺]_i) in Purkinje cells, we tested the hypothesis that postsynaptic Ca²⁺ is involved in triggering rebound potentiation. When Purkinje cells were filled with an internal solution containing the Ca²⁺ chelator BAPTA, climbing fibre stimulation (five pulses at 0.5 Hz) failed to induce a potentiation of i.p.s.cs, but depressed their amplitudes (Fig. 1b). Furthermore, direct activation of voltage-gated Ca²⁺ channels by a depolarizing pulse to 0 mV for 500 ms from a holding potential of −60 or −70 mV¹⁶ induced a prolonged potentiation of i.p.s.cs (Fig. 1c, and see summary in Fig. 4) to an extent and with a time course similar to that of climbing fibre-induced rebound potentiation (in the range of several tens of minutes). In control experiments, Na⁺ action potentials were evoked antidromically by stimulating Purkinje cell axons in the granule cell layer¹² with a stimulation

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pattern (five pulses at 0.5 Hz) similar to that used for the activation of climbing fibres. This activation of antidromic spikes did not induce a rise in $[Ca^{2+}]_i$ (not shown) or a potentiation of i.p.s.c.s (Fig. 1d). By contrast, the usual 'run-down' of i.p.s.c. amplitudes was seen (Fig. 4). Taken together these findings strongly suggest that a climbing fibre-induced rise in $[Ca^{2+}]_i$ through voltage-gated Ca^{2+} channels initiates the induction of rebound potentiation.

To investigate more directly the mechanisms underlying rebound potentiation we combined whole-cell patch-clamp recordings, fluorometric $[Ca^{2+}]_i$ measurements and bath-applications of exogenous GABA in the same experiment (Figs 2 and 3). Purkinje cells were filled with the Ca^{2+} indicator dye Fura-2 (200 μ M) through the patch-pipette and $[Ca^{2+}]_i$ was continuously monitored by digital fluorescence video ratio imaging¹⁶. Figure 2 shows the results from such an experiment. In parallel to the monitoring of rebound potentiation of i.p.s.c.s induced by climbing fibre stimulation (Fig. 2a), bath-applied GABA currents (2 μ M for 10 s) were measured intermittently (Fig. 2b). After climbing fibre stimulation these currents induced by exogenous GABA application were also strongly enhanced with a time course of potentiation which was very similar to that observed for the potentiation of the i.p.s.c.s (Figs 2a, b). This indicates that the rebound potentiation involves an up-regulation of the postsynaptic GABA_A receptor function. As in the control experiments for i.p.s.c.s, after antidromic stimulation (five pulses, 0.5 Hz), responses to bath-applied GABA were not potentiated, but became smaller (Fig. 4).

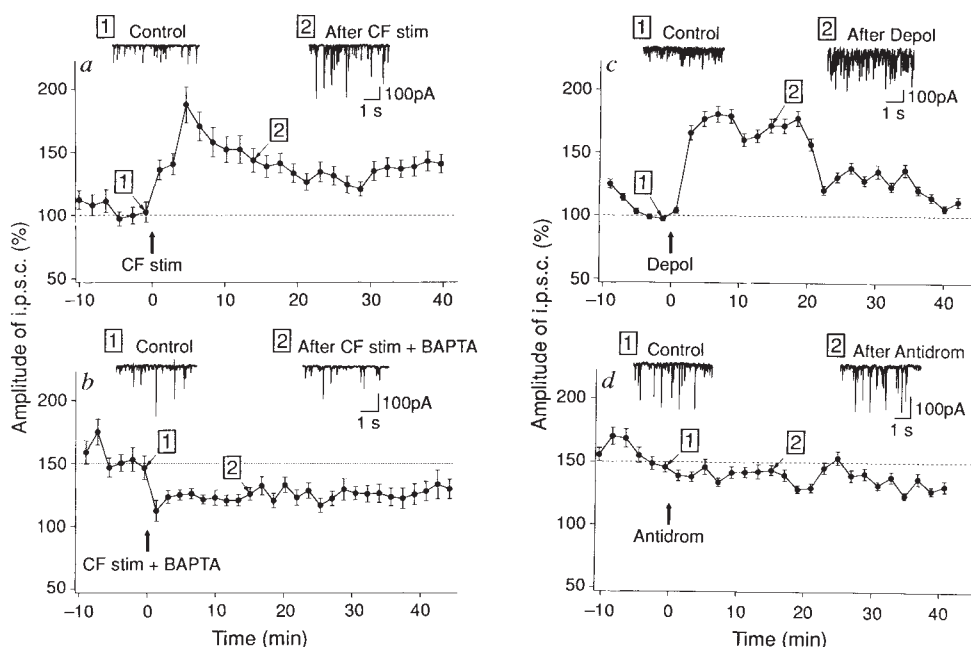
Figure 2c shows that the basal level of $[Ca^{2+}]_i$ in this cell had quite similar values in the soma and in the dendrites of around 100 nM (see also Fig. 3a). On the conditioning climbing fibre

stimulation, the dendritic $[Ca^{2+}]_i$ rose steeply to about 900 nM (Fig. 2c), decaying rapidly back to about 30 nM of its baseline after ~40 s. The $[Ca^{2+}]_i$ in the somatic region rose only slightly to <200 nM (Figs 2c and 3b). A similar pattern of $[Ca^{2+}]_i$ changes was observed in all five cells tested. Note that the $[Ca^{2+}]_i$ in both regions of the Purkinje cells, dendrites and soma maintained, after the transient rise, a stable baseline value with slow fluctuations of less than 20–30 nM (Fig. 2c), whereas the simultaneously measured i.p.s.c.s and GABA-induced currents were markedly potentiated (Fig. 2a, b). These results indicate that the potentiation of i.p.s.c.s is triggered by a transient rise in $[Ca^{2+}]_i$ in the Purkinje cell dendrites, and that the maintenance of the potentiation is not due to a persistent elevation of $[Ca^{2+}]_i$ after climbing fibre stimulation. In fact, in Purkinje cells a constant, high $[Ca^{2+}]_i$ of 500–1,000 nM produces a depression rather than a potentiation of responses to applied GABA¹⁷.

Llano *et al.*¹⁸ reported that Ca^{2+} entry due to depolarization induces in Purkinje cells a short-lasting depression of i.p.s.c.s which they attributed to a presynaptic mechanism. In our experiments, climbing fibre stimulation also induced a short-lasting (about 30–40 s) depression of the i.p.s.c. amplitudes (not shown) which seems to be caused by the elevation of $[Ca^{2+}]_i$ to values in the micromolar range¹⁷ and thus is of postsynaptic origin. In contrast to the decrease in frequency of the i.p.s.c.s observed during the depolarization-induced depression¹⁸, during rebound potentiation no significant change in i.p.s.c. frequency could be detected. The relative frequencies of i.p.s.c.s obtained 20 min after conditioning (in per cent of the control values, mean $\pm$ s.d.) were: $95 \pm 15\%$ ($n=5$) for climbing fibre stimulation, $105 \pm 19\%$ ($n=4$) for depolarization, $99 \pm 11\%$ ($n=6$) for climbing fibre stimulation with intracellular BAPTA, and

FIG. 1 Rebound potentiation (RP) of i.p.s.c.s in cerebellar Purkinje cells (PCs). *a*, Climbing fibre (CF) stimulation (5 pulses, 0.5 Hz) induces prolonged potentiation of i.p.s.c.s. *b*, CF stimulation (5 pulses, 0.5 Hz) fails to induce potentiation when the intracellular solution contained 30 mM BAPTA, instead a reduction of i.p.s.c.s amplitudes was observed. Note that this relatively high concentration of BAPTA was chosen to be able to block the rise in $[Ca^{2+}]_i$ within 15 min of starting the whole-cell recording. Lower concentrations (5–10 mM) can also be used, but because of the long diffusion time in PCs with an extended dendritic tree, more time is needed for remote sites to reach the effective concentration¹⁸. *c*, Depolarization of PCs (single pulse from holding potential -60 or -70 mV to 0 mV, 500 ms) induced potentiation of i.p.s.c.s. *d*, Activation of antidromic spikes (5 spikes, 0.5 Hz) does not potentiate i.p.s.c.s. Each point represents the mean $\pm$ s.e.m. of amplitudes of 100–200 consecutive i.p.s.c.s normalized to the values before conditioning. Because there is a tendency for the amplitudes of i.p.s.c.s to decay slowly during the control period, the last three values before conditioning were chosen for calculating the baseline (broken line at the 100% level). The threshold for detecting the i.p.s.c.s was set to -50 pA, so that the vast majority of miniature i.p.s.c.s were not counted³⁰. Current traces are taken before (1) and 14 min after (2) conditioning. The traces in the insets were recorded at the time points indicated with thin arrows. The upward thick arrow indicates the time of conditioning.

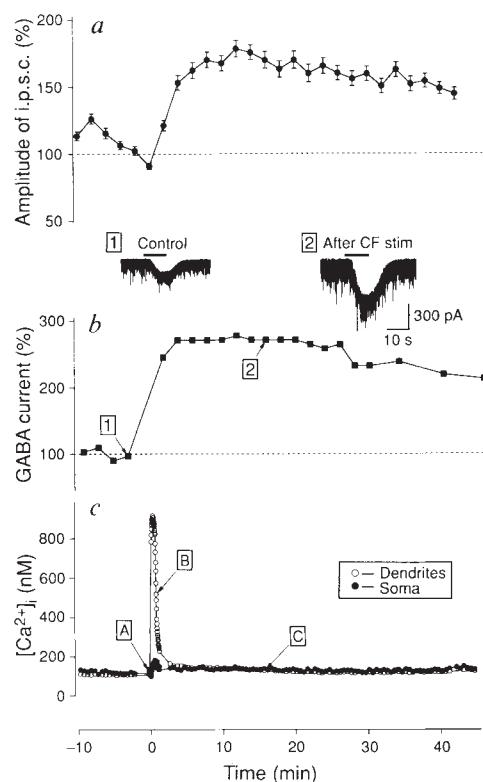
METHODS. Sagittal slices of 200 μ m were prepared from the cerebellum of 12–16-day old rats as described previously³⁹. Whole-cell patch-clamp recording methods were used to record synaptic currents in PCs directly visualized through a X40 water immersion objective in an upright microscope (Zeiss Axioscope). Tight-seal whole-cell recordings (seal resistances greater



than 10 G Ω) were made with patch pipettes of resistances 2–4 M Ω (ref. 7). In all experiments, series resistance was compensated using the standard procedure of the EPC-7 amplifier (List Electronics, Darmstadt, Germany). The standard internal solution contained (in mM): 75 CsCl, 2 MgCl₂, 80 D-glucuronate, 1 EGTA, 4 Na-ATP, 0.4 Na-GTP and 10 HEPES (pH 7.3, adjusted with CsOH). In experiments with BAPTA, the composition of the internal solution was (in mM): 40 CsCl, 2 MgCl₂, 50 D-glucuronate, 1 EGTA, 4 Na-ATP, 0.4 Na-GTP and 30 BAPTA (pH 7.3, adjusted with CsOH). The experimental chamber was continuously perfused with a saline containing (in mM) 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃ and 20 glucose, which was bubbled continuously with a mixture of 95% O₂ and 5% CO₂. All experiments were at room temperature (22–24 $^{\circ}$ C).

FIG. 2 The transient $[Ca^{2+}]_i$ rise accompanying the rebound potentiation (RP) of i.p.s.cs and the potentiation of current responses to bath-applied GABA. **a**, RP of i.p.s.cs after climbing fibre (CF) stimulation (5 pulses, 0.5 Hz). **b**, Potentiation of Purkinje cell (PC) current responses to bath-applied GABA (2 μ M, 10 s). Insets display current traces recorded before (1) and 15 min after (2) CF stimulation. GABA was applied during the period indicated by the horizontal bars on top of each trace. **c**, $[Ca^{2+}]_i$ in the soma and the dendrites. The regions from which the integral of $[Ca^{2+}]_i$ changes were measured are indicated in the digital fluorescent ratio images (DFI) in Fig. 3. The points A, B and C mark the time at which the DFI shown in Fig. 3 were taken.

METHODS. For measuring the $[Ca^{2+}]_i$, the pentapotassium salt of the Ca^{2+} indicator Fura-2 (200 μ M) was added to the internal solution. Changes in $[Ca^{2+}]_i$ were monitored using a digital imaging system (VPROBE, ETM-Systems, Mission Viejo, California). Background images of the preparation were constructed from the 360:380 nm fluorescence ratios while the pipette was in the cell-attached mode. After establishment of the whole-cell configuration, paired exposures to 360 and 380 nm were used to construct background-corrected DFI. The DFI were taken at a maximum rate of 1 per s. They were displayed on-line as pseudocolour images on a monitor and stored on hard-disk for later analysis. The gain (corresponding to the dynamic range of the system in this case) of the intensified video CCD camera (Hamamatsu) was set at values that optimized detection of signals from the dendritic arborization. At the gain selected in this experiment, the signal from most of the soma was saturated, which is indicated by the darkened area in the soma in the DFIs (Fig. 3). But signals near the membrane of the soma can be approximated by measuring in the non-saturated sites at the edge. The $[Ca^{2+}]_i$ was calculated according to Grynkiewicz *et al.*³¹. The parameters K_d , R_{min} and R_{max} characterizing the system were 1,938 nM, 0.28 and 7.89 respectively. These parameters correspond to average values obtained from *in vitro* calibrations. Using a photomultiplier system³², comparisons were made of the parameters obtained from *in vitro* calibrations and those obtained from *in situ* calibrations for PCs. These comparisons indicate that *in vitro* calibrations lead to an underestimation of the absolute values of $[Ca^{2+}]_i$, the error being roughly 2–5%. This error was not corrected for the data presented. Another source of error that was left uncorrected is related to the observation that strong ultraviolet illumination may cause slow changes in the background fluorescence of the slice.



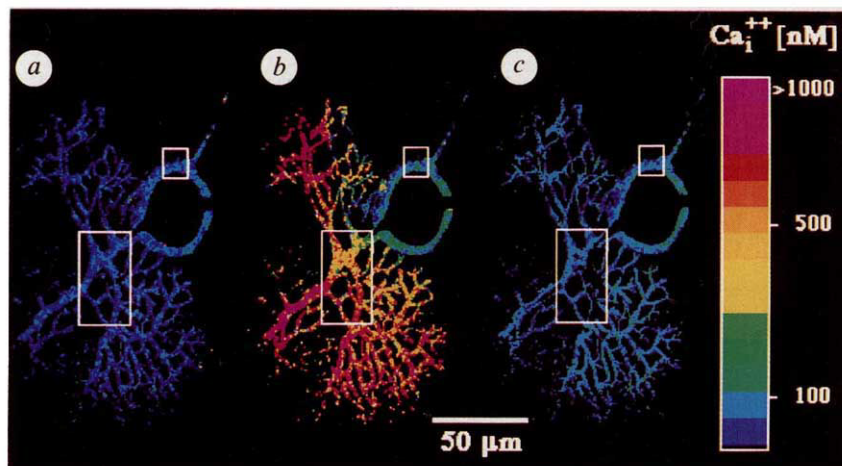
100 $\pm$ 3% ($n=3$) for the control experiments with antidromic stimulation.

Note that with climbing fibre stimulation (Figs 2 and 4) there is a larger potentiation of GABA-induced currents compared with the magnitude of rebound potentiation of i.p.s.cs. Differences in agonist concentrations, mode of agonist application and receptor kinetics may account for the different degrees of potentiation. But the similarities in the mode of induction (climbing fibre stimulation and/or depolarization¹⁸) and in time course (Figs 2 and 4), as well as the failure of antidromic stimulation to induce potentiation (Fig. 4), suggest that the same mechanism, namely an upregulation of postsynaptic GABA_A receptor function, is responsible for the potentiation of both types of current responses.

The delay of 3–15 min between the transient increase in

$[Ca^{2+}]_i$ and the peak of potentiation (Fig. 2) strongly suggests a contribution of intracellular second messengers for the establishment of potentiation. For example, the protein kinase A second messenger system can reversibly potentiate the Purkinje cell responses to GABA (ref. 19, and M.K. and A.K., manuscript in preparation). But because there is no evidence so far for a direct relation between $[Ca^{2+}]_i$ and protein kinase A in Purkinje cells, other Ca^{2+} -activated intracellular processes, such as those involving protein kinase C and/or protein kinase G, must also be considered. The effects of GABA responses observed in the Purkinje cells after an increase in $[Ca^{2+}]_i$ (ref. 18) are opposite to those reported for hippocampal, spinal cord, habenular and dorsal root ganglion neurons (refs 20–22, and M.K. and A.K., unpublished results). In these cells there was a depression rather than a potentiation of GABA responses. The effect of protein

FIG. 3 Digital fluorescence images (DFI) of $[Ca^{2+}]_i$ in a PC before (a) during (b) and after (c) CF stimulation. DFI were taken at the time points indicated in Fig. 2c by a, b and c, respectively. The rectangular white boxes drawn on the images delimit the regions from which the integral of the $[Ca^{2+}]_i$ changes were calculated. Same experiments as in Fig. 2.



kinase A activation in hippocampal and spinal cord cells is also opposite to that seen in cerebellar Purkinje cells^{23,24}. In the intermediate lobe cells of the pituitary gland, GABA_A receptors are modulated in a complex way by $[Ca^{2+}]_i$ changes after the release of Ca^{2+} from intracellular stores²⁵. Despite the amount of data, the mechanism responsible for the upregulation of the GABA_A receptor function by second messengers leading to rebound potentiation is not understood.

Does the rebound potentiation in cerebellar slices have any physiological significance? Experiments *in vivo* demonstrate a strong dependence of Purkinje cell excitability on the presence of active climbing fibre synapses. Thus, a marked increase in cell excitability was observed in experiments in which 'spontaneous', recurrent firing of climbing fibres was suppressed^{5,6}. Moreover, after destruction of the inferior olive, the brain structure from which the climbing fibres originate, this effect is reported to last for up to 30 days²⁶. Our results provide a likely mechanism for this phenomenon. We hypothesize that *in vivo*, $[Ca^{2+}]_i$ changes caused by spontaneously occurring climbing fibre signals regulate the GABA_A receptor function, thereby controlling the Purkinje cell's excitability. Normally, spontaneously occurring climbing fibre activity will maintain the GABA_A receptor sensitivity at a high level. But in the absence of climbing fibre activity, GABA_A receptor sensitivity and, therefore, the efficacy of inhibitory synapses decreases causing the observed increase in excitability of Purkinje cells^{5,6}.

In addition to tonically depressing the Purkinje cell excitability, climbing fibre stimulation can induce long-term depression of parallel fibre excitatory synapses, when these inputs are activated in conjunction with climbing fibres, a phenomenon presumed to be a cellular mechanism underlying motor learning (for review, see ref. 4). Sustained desensitization of postsynaptic AMPA receptors at parallel fibre synapses is thought to mediate long-term depression²⁷⁻²⁹. It thus seems that climbing fibre signals regulate the excitability of Purkinje cells through the up- and downregulation of postsynaptic GABA_A and AMPA receptors, respectively. In concert with long-term depression of excitatory parallel fibre synapses, rebound potentiation of inhibitory

synapses found here may have an important role for motor learning in the cerebellum. □

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Tumour necrosis factor α restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice

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SCHISTOSOMIASIS (bilharzia) is a parasitic disease caused by several species of schistosome worms (blood flukes). The key pathogenic event in this disease is the formation of granulomas around schistosome eggs trapped in portal venules of the liver¹⁻⁹. Granulomas are a distinctive form of chronic inflammation characterized by localized aggregation of activated macrophages around an inciting stimulus¹⁰. Each granuloma evolves to form a fibrous scar; in schistosomiasis, the result is widespread hepatic fibrosis and portal hypertension. To identify the specific immune signal molecules necessary for granuloma formation, we studied schistosome infections in severe combined immunodeficient (SCID) mice, which have normal macrophages but lack functional B or T lymphocytes^{11,12}. Here we report that the immunoregulatory cytokine tumour necrosis factor α is necessary and sufficient to reconstitute

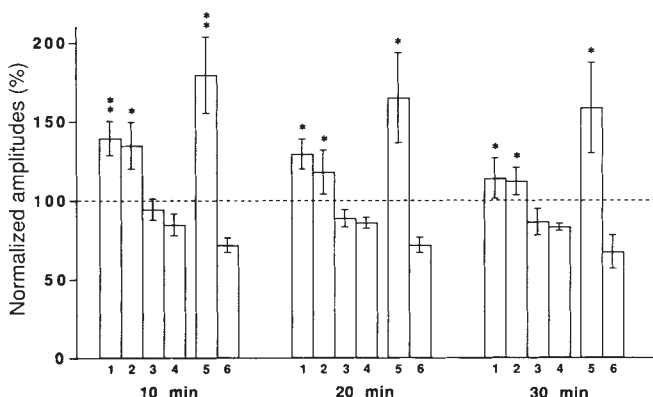


FIG. 4 Average changes in i.p.s.c. amplitudes (bars 1-4) and whole-cell current responses to bath-applied GABA (bars 5 and 6) produced by several experimental manipulations: (1) CF stimulation (5 pulses at 0.5 Hz) ($n=6$); (2) a depolarizing pulse from the holding potential of -60 or -70 mV to 0 mV for 500 ms ($n=4$); (3) CF stimulation (5 pulses at 0.5 Hz) in the presence of 30 mM BAPTA in the recording pipette ($n=6$); (4) antidromic stimulation (5 pulses at 0.5 Hz) ($n=4$); (5) CF stimulation for GABA-induced whole-cell current responses ($n=5$); (6) antidromic stimulation for GABA-induced whole-cell current responses ($n=4$). Values shown represent the percent mean ($\pm$ s.e.m.) changes relative to the control values before conditioning. The changes were measured at 10, 20 and 30 min after conditioning. Statistical significance tests were done by comparing at each time point, respectively, the i.p.s.c. amplitudes of bars 1, 2, 3 and to those of bar 4, and the exogenous GABA-induced whole-cell current responses of bar 5 and to those of bar 6 (Mann-Whitney U -test, ** $P < 0.01$, * $P < 0.05$). Note that at each point (10, 20 and 30 min) bars 3 were not significantly different from bars 4.

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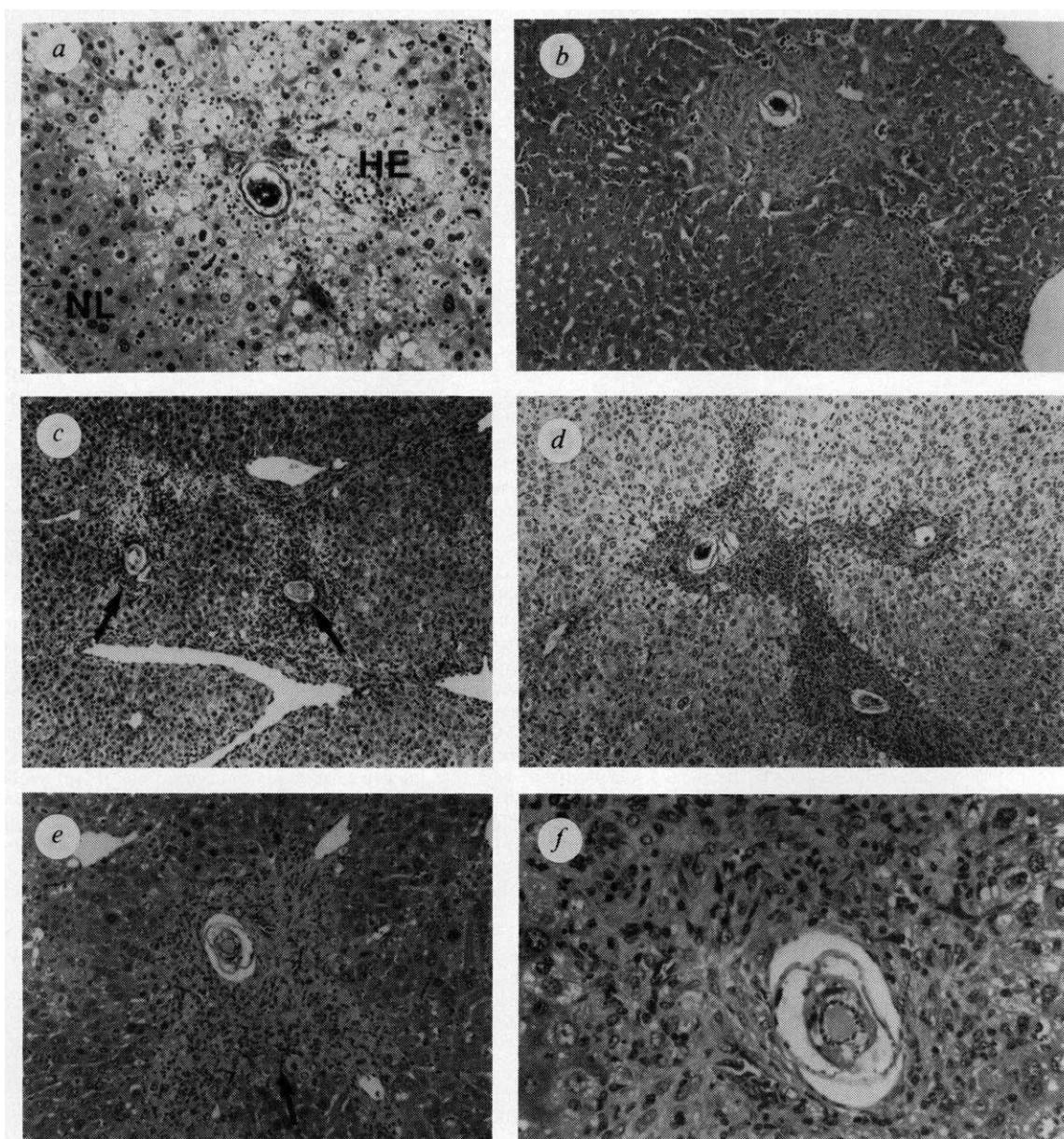


FIG. 1 Cellular reaction to schistosome eggs in SCID mice with and without immune reconstitution. Sections show eggs in liver stained by haematoxylin and eosin eight weeks after infection of mice with cercariae. Eggs containing embryos (miracidia) are seen in each panel and are $120 \times 60 \mu$. a, SCID mouse with no reconstitution. Note hepatotoxic effect (HE) in vicinity of egg. Hepatocytes are swollen and appear vacuolated on haematoxylin and eosin stain. Oil red O stain (not shown) indicates that the vacuoles in hepatocytes are accumulations of lipid. In lower left are normal hepatocytes (NL) for comparison. Note lack of granulomatous inflammation surrounding egg. b, BALB/c mouse showing typical granulomatous reaction and lack of hepatotoxic effect. c, SCID mouse injected with $0.5 \mu\text{g}$ TNF α at seven weeks post-infection and examined one week later. Eggs are indicated by arrows.

granuloma formation in schistosome-infected SCID mice. Moreover, we find that the parasitic worms require tumour necrosis factor α for egg-laying and for excretion of eggs from the host. The implication of this latter result is that the parasite has adapted so successfully to its host that it uses a host-derived immunoregulatory protein as a signal for replication and transmission.

SCID mice infected with *Schistosoma mansoni* cercariae supported normal parasite development and mating, but did not produce granulomas in response to eggs trapped in the liver at eight weeks post-infection (Fig. 1a). Compared with control immunocompetent BALB/c mice, schistosome-infected SCID

Note early granuloma formation and some diminution in hepatotoxic effect compared to a. d, SCID mouse reconstituted with activated T-cell supernatant at seven weeks post infection. Note well-formed granulomas and minimal hepatotoxic effect at border of granuloma (compare with a). e, SCID mouse reconstituted with $5 \mu\text{g}$ TNF α at seven weeks post-infection. Note well-formed granuloma and minimal hepatotoxic effect at border of granuloma. f, Higher magnification of e to illustrate cellular infiltrate around egg. Note presence of cells with large, clear nuclei and abundant cytoplasm ('epithelioid' macrophages) as well as cells with smaller, darker nuclei (lymphocytes and occasional neutrophils). Staining of sections with reticulum stain for type III collagen showed concentric fibres around larger granulomas consistent with early fibrosis (not shown).

mice had more adult worm pairs, yet the number of eggs produced by these worm pairs was reduced by $>70\%$ (Table 1) and no eggs were excreted in the stools of infected SCID mice. Most eggs trapped in the liver of SCID hosts produced no inflammatory reaction (Table 1), and the remainder evoked only small abscess-like collections composed predominantly of neutrophils. A more acute hepatic disease was observed, associated with a mortality of 30% at 10 weeks post-infection (versus no mortality at 10 weeks in BALB/c mice). Mortality correlated with hepatic failure due to severe hepatotoxic damage around schistosome eggs (Fig. 1a).

TABLE 1 Parasite egg counts and granuloma formation in SCID mice, BALB/c mice and reconstituted SCID mice

	Adult worm pairs	Total eggs in liver	Eggs with granuloma (%)	No. of eggs in the stool per day	Volume of granuloma (mm ³ × 10 ²)
SCID mice (n=5)	12 ± 7	7,000 ± 900	7 ± 2	0	0.04 ± 0.01
BALB/c mice (n=7)	5 ± 2	28,000 ± 800	99 ± 1	250	5.0 ± 1.0
SCID mice reconstituted with:					
Spleen cells from infected mice (n=3)	16 ± 6	17,000 ± 1,700	83 ± 5	ND	3.5 ± 1.0
Spleen cells from uninfected mice (n=3)	18 ± 4	12,000 ± 1,300	25 ± 5	ND	2.0 ± 0.1
Serum from infected BALB/c (n=4)	ND	14,600 ± 2,300	62 ± 5	ND	0.5 ± 0.2
Serum from infected BALB/c (n=3) treated with anti-TNF α	ND	8,300 ± 1,900	47 ± 2	ND	0.2 ± 0.04
Activated T-cell medium (n=7)	9 ± 6	16,000 ± 2,000	76 ± 13	ND	1.2 ± 0.3
Activated T-cell medium treated with anti-TNF α (n=2)	10 ± 2	6,700 ± 1,800	8 ± 1	ND	0.4 ± 0.1
Activated T-cell medium, treated with anti-IL-4 and anti-IL-5 (n=2)	ND	14,500 ± 4,000	76 ± 12	ND	2.2 ± 1.0
TNF α 0.05 μ g (n=2)	ND	6,000 ± 1,000	12 ± 2	ND	0.04 ± 0.02
0.5 μ g (n=2)	ND	12,000 ± 450	29 ± 3	0	1.0 ± 0.4
5 μ g (n=8)	7 ± 2	21,500 ± 7,000	80 ± 3	12	3.3 ± 0.2

Homozygous SCID mice were maintained under aseptic conditions in filter-top cages, with sterile food, water and bedding. Complete post mortem examination of all subject animals revealed no intercurrent diseases. Syngeneic BALB/c mice (5–7 weeks of age) were injected subcutaneously with 55–60 cercariae of *S. mansoni* (Puerto Rican strain) shed from infected *Biomphalaria glabrata* snails. For the syngeneic spleen cell transplants, two infected (7 weeks post-exposure to *S. mansoni* cercariae) and 2 uninfected BALB/c mice were killed and their spleens removed under sterile conditions. Each spleen was minced in 5.0 ml sterile PBS containing 100 μ g gentamicin. Cells were allowed to disperse for a few minutes at room temperature. The mixture was centrifuged at 200g for 5 min. The supernatant was removed and the pellet washed twice with 5.0 ml PBS before resuspension in 1.0 ml PBS. Infected SCID mice (seven weeks post-exposure to *S. mansoni* cercariae) were injected interperitoneally with 10⁷ cells from the spleen preparation of the infected or uninfected BALB/c mice. SCID mice were killed 10–14 days post injection. Reconstitution of SCID mice with immune cell products were carried out during the seventh week of infection of scid mice. All injections were interperitoneal, and all animals were assayed for number of adult worms, eggs and size of granuloma in the liver 10–14 days post-reconstitution. Doses given are the amount injected per mouse: 100 μ l serum from BALB/c mice infected with *S. mansoni*, 100 μ l serum treated with 1.0 μ l of anti-murine TNF α monoclonal antibody at a concentration of 5.75 mg ml⁻¹, 300 μ l medium from 10⁶ D10.G4.1 cells ml⁻¹ conditioned with 1 mg ml⁻¹ conalbumin and 5 × 10⁴ irradiated syngeneic spleen cells for 72 h, 300 μ l T-cell medium incubated with anti-murine IL-4 (monoclonal antibody 11B11, rat IgG1) and anti-murine IL-5 (monoclonal antibody TRFK-5, rat IgG1), 300 μ l T-cell medium incubated with anti-TNF α monoclonal antibody (TN3-19.12, hamster IgG; Genentech), or 0.05 to 5.0 μ g of recombinant murine TNF α (Genentech) with a specific activity of 1.2 × 10⁷ units mg⁻¹. Individual mice at the eighth week of infection were isolated in separate cages for a 24 h period to collect faeces. Faeces were mixed with 5.0 ml 10% formalin and allowed to fix for 4 h. The mixture was passed through 150- μ m wire mesh to remove debris. Four 250- μ l fractions of each preparation were taken and *S. mansoni* eggs were counted. The numbers reported are the mean number of eggs passed per mouse per 24 h. Adult worms were recovered by perfusion of the portal venous system²⁵. Mice were killed by interperitoneal injection of 50 mg sodium barbital and 600 units of heparin in 500 μ l PBS. numbers reported are the mean number of adult worms per mouse per experiment ± standard deviation. To quantify the number of parasite eggs in the liver, the liver was homogenized for 20–30 seconds in 25 ml of Earl's basic salt solution (Gibco BRL) and trypsin (1% solution) using a Brinkman PCU- α homogenizer. The mixture was incubated at room temperature for 3–4 h then shaken well and 20- μ l samples removed. Eggs were counted under a microscope. Ten different samples were independently counted for each liver. The numbers reported are the mean total number of eggs per liver per experiment ± standard deviation. To evaluate granuloma size and fibrosis all major organs were removed from mice, fixed for 12 h in 10% phosphate-buffered formalin and embedded in paraffin. Paraffin sections were cut at 5 μ m and stained with haematoxylin and eosin. The total number of eggs and the number of eggs with and without granuloma were counted. The volume of granulomas was calculated²⁶. The mean of the two numbers were used for calculations of the volume assuming a spherical shape for the granulomas. Volumes reported are the mean of 10 independent calculations. *n*, Number of individual mice assayed per experiment. Data is mean ± standard deviation for *n* ≥ 3 or ± standard error for *n* = 2. ND, not determined.

To identify the host factors required for granuloma formation, a series of reconstitution experiments was performed using sera or spleen cells from major histocompatibility complex (MHC)-compatible BALB/c mice. Spleen cells from infected BALB/c mice, when injected into SCID mice (seven weeks post-infection), resulted in reconstitution of a granulomatous reaction to schistosome eggs which was nearly identical to that of immune-competent mice (Table 1). Spleen cells from uninfected BALB/c mice and sera from infected animals were less effective in reconstituting granuloma formation (Table 1).

Schistosome eggs are a potent stimulus for clonal expansion and activation of the Th-2 class of CD4⁺ cells, which release specific cytokines^{13,14}. Culture supernatants enriched in these cytokines were obtained by stimulating the conalbumin-specific Th-2 cell clone D10.G4.1 (refs 15, 16) with specific antigen in the presence of conditioned media from concanavalin A-stimulated spleen cells. When injected into schistosome-infected SCID mice, these crude Th-2 supernatants yielded a granulomatous reaction identical to that produced by spleen cells from an infected BALB/c donor (Table 1; Fig. 1b and d). To identify the specific component that evoked granuloma formation, the supernatants were preadsorbed with antisera against various Th-2 cell products. Depletion of interleukin-4 (IL-4) and IL-5 had no detectable effect, but preadsorption with a monoclonal antiserum specific for tumour necrosis factor α (TNF α) inhibited the granulomatous response (Table 1).

Indeed, injection of purified recombinant TNF α alone led to a dose-dependent formation of granulomas around schistosome eggs (Table 1; Fig. 1c, e, f). This cytokine is thus sufficient to restore granuloma formation in schistosome-infected SCID mice. Reticulum staining of TNF α -induced granulomas indicated that recruitment of fibroblasts and deposition of collagen fibres took place within the granuloma.

Treatment with TNF α produced a dose-dependent increase in the total number of parasite eggs recoverable in the liver from infected SCID hosts (Table 1). A similar effect was observed with sera from some of the infected BALB/c mice, and could

TABLE 2 Effect of TNF α on egg-laying by *S. mansoni* female worms *in vitro*

Concentration of TNF α (ng ml ⁻¹)	0	10	20	40
Number of female worms	14	13	14	11
Number of eggs per 72 h	16	55	89	159
Mean no. of eggs per female	1.1	4.2	6.4	14.5

Female worms were dissected from the mesenteric veins of Syrian golden hamsters infected seven weeks previously with *S. mansoni* cercariae. Worms were maintained in culture as described²⁷. TNF α had no effect on motility, survival or growth of the worms, either acutely or when monitored for an additional two months. When a second dose of TNF α was added to these cultures one week later, a second burst of egg-laying occurred (data not shown). Data are cumulative from four independent assays at each concentration.

be abolished by pretreatment of these sera with a monospecific antiserum against TNF α (Table 1). This suggests that TNF α could directly stimulate egg production by adult schistosomes. To test this hypothesis, adult worm pairs were isolated from hamsters seven weeks after infection, and were maintained in culture in the presence or absence of recombinant human TNF α . A dose-dependent 15-fold increase in egg-laying was apparent within 72 h after TNF α treatment (Table 2).

Several conclusions can be drawn from these results. First, TNF α plays an essential role in mediating granuloma formation in response to schistosome ova. This is consistent with evidence that circulating and granuloma TNF α levels are significantly elevated in schistosome infections¹⁷ and that selective depletion of TNF α inhibits the granulomatous response to *Mycobacterium bovis*¹⁸. In an immunocompetent host, the induction of granulomas probably involves a number of discrete immunoregulatory processes, including macrophage chemotaxis, localized activation of macrophages and endothelial cells, recruitment and activation of T lymphocytes, and initiation of secondary lymphokine cascades. TNF α stimulates endothelial cells to express leukocyte adhesion molecules, induces maturation of monocytes and multinucleated giant cells, and induces fibroblast mitosis¹⁹. The schistosome egg antigens are a second key component that localizes the effects of TNF α around schistosome eggs. We hypothesize that the schistosome egg surface or antigens released locally prime endothelial or inflammatory cells so that antigen-sensitized T cells could supply TNF α with the subsequent production of additional granuloma mediators. Amplification of this reaction could then occur through release of TNF α by macrophages recruited into the granuloma, as has been proposed for the granulomatous response to *M. bovis*¹⁸. The mitogenic effect of TNF α on fibroblasts could be in part responsible for the initial stages of fibrosis of the granuloma, as has been proposed for the development of silica-induced pulmonary fibrosis²⁰. Further analysis of TNF α -dependent granuloma formation in SCID mice may shed light on the pathogenesis of schistosomiasis, other granulomatous infections such as tuberculosis, brucellosis, syphilis, leprosy, and deep fungal infections, and granulomatous diseases of unknown aetiology, such as Crohn's disease and sarcoidosis.

Compounds capable of inhibiting TNF α activity could be useful for the treatment of schistosomiasis and other disorders in which granulomatous inflammation is a major source of morbidity. This approach would require effective measures to protect the host against the direct toxic effect of schistosome eggs on the liver. The absence of a granulomatous response leaves the host vulnerable to acute, severe liver damage caused by a diffusible hepatotoxin elaborated by the eggs^{7,21,22}. Granuloma formation and the consequent perioval fibrosis appear to benefit the host by sequestering and/or inactivating this toxin. At the same time, prolonged host survival increases the opportunity for egg production by the parasite, increasing the chance of its transmission to new hosts²².

The host-parasite relationship in schistosomiasis is more intricate than previously realized. Egg laying by adult females is significantly stimulated by the presence of TNF α . This observation is interesting because schistosome worm pairs reside in the portal venous system, where TNF α levels could be elevated as a result of stimulation of macrophages and endothelial cells by gut-derived endotoxin. Furthermore, the granulomatous reaction itself, by virtue of its degradative enzymes, may be necessary for passage of the immobile parasite eggs through the wall of the intestine or bladder to allow excretion from the host^{23,24}. Thus a host immune response, evolved as protection against infection, induces parasite replication and transmission. □

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CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones

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OCCUPANCY of the T-cell antigen receptor is insufficient to induce T-cell activation optimally; a second co-stimulatory signal is required¹. Exposure of T-cell clones to complexes of antigen with major histocompatibility complex molecules in the absence of the co-stimulatory signal induces a state of clonal anergy. This requirement for two stimuli for T-cell activation could have an important role *in vivo* in establishing peripheral tolerance to antigens not encountered in the thymus^{1,2}. The receptor on T cells required for the co-stimulatory stimulus involved in the prevention of anergy has not been identified. The human T-cell antigen CD28 provides a signal that can synergize with T-cell antigen receptor stimulation in activating T cells to proliferate and secrete lymphokines³⁻⁶. Here we report that a monoclonal antibody against the murine homologue of CD28 (ref. 7; J.A.G. *et al.*, manuscript in preparation) can provide a co-stimulatory signal to naive CD4⁺ T cells and to T-cell clones. Moreover, we demonstrate that this co-stimulatory signal can block the induction of anergy in T-cell clones.

To examine the requirements of CD4⁺ T cells for co-stimulation, the response of these cells when exposed to T-cell antigen receptor (TCR) signals provided by immobilized anti-CD3 antibody alone or with various additions, was determined. No proliferation occurred when the cells were cultured on plates coated with <2.5 $\mu\text{g ml}^{-1}$ anti-CD3, although proliferation was obtained at higher anti-CD3 levels (Fig. 1a). Vigorous

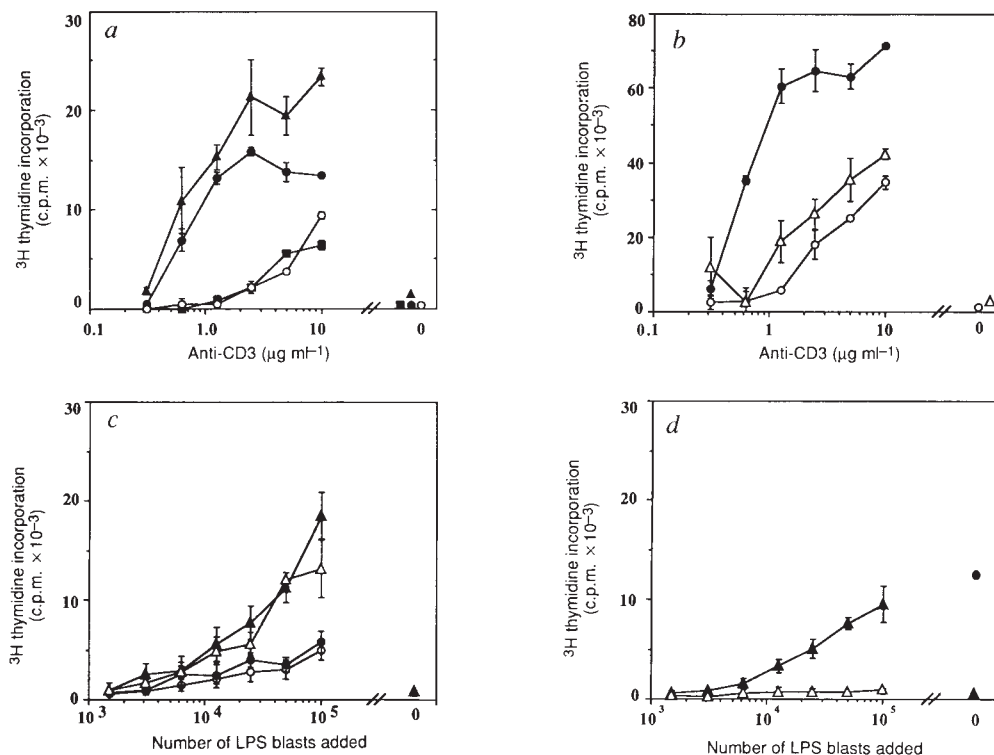
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FIG. 1 A CD28-mediated interaction is necessary for optimal proliferation of murine CD4⁺ T cells. *a*, Co-stimulation of CD4⁺ T cells by LPS-induced syngeneic blasts (▲) or anti-CD28 (●) exhibit the same kinetics. Syngeneic blasts were added at 5×10^5 per culture. ■, Response of CD4⁺ T cells on anti-CD3-coated plates. A control hamster antibody, F531 (○), had no effect. Thymidine incorporation by CD4⁺ T cells in the absence of anti-CD3 was 324 ± 35 c.p.m.; plus anti-CD28 only, 332 ± 79 c.p.m.; plus F531, 280 ± 117 ; plus blasts $3,595 \pm 445$ c.p.m. *b*, Co-stimulation of CD4⁺ T cell proliferation by anti-CD28 is distinct from the effects of anti-Thy-1. CD28 ascites at a dilution of 1:500 (●), anti-Thy-1 antibody G7 supernatant⁸ at 25% final concentration (△), or medium alone (○), were added to CD4⁺ T cells on plates coated with anti-CD3. G7 supernatant in the absence of anti-CD3 resulted in thymidine incorporation of $3,018 \pm 1,174$ c.p.m. *c*, Co-stimulation induced by syngeneic LPS blasts can be blocked by anti-CD28 Fab fragments. Plates were coated with a $1 \mu\text{g ml}^{-1}$ solution of anti-CD3. Syngeneic blasts were added at the indicated number per well. Cultures contained no additional antibody (▲), $50 \mu\text{g ml}^{-1}$ F531 Fab (△), $10 \mu\text{g ml}^{-1}$ anti-CD28 Fab (●), or $50 \mu\text{g ml}^{-1}$ anti-CD28 Fab (○). Thymidine incorporation by CD4⁺ T cell cultures with no added blasts was 473 ± 116 c.p.m. *d*, Co-stimulatory activity of LPS blasts is destroyed by fixation with ECDI. Unfixed (▲) or ECDI-fixed (△) LPS blasts were added to plates coated with $1 \mu\text{g ml}^{-1}$ anti-CD3, followed by CD4⁺ T cells. Filled triangle at 0 indicates thymidine incorporation by T cells alone; filled circle indicates incorporation by T cells plus 1:500 dilution of anti-CD28 ascites. METHODS. Lymph node cells from 4–8-week-old BALB/c mice were passed over nylon wool, then treated sequentially with anti-class II MHC (28–16–8s) and anti-CD8 (3.155) plus complement. Resultant purity was >95% CD4⁺



cells. Round-bottomed 96-well plates were coated with anti-CD3 (500A2) in HBSS for 2 h at 37 °C. Plates were washed extensively with HBSS, then blocked with RPMI 1640 medium containing 10% FCS. Anti-CD28 and control hamster ascites F531 were used at a final dilution of 1:500. CD4⁺ T cells were added at 5×10^4 per well in RPMI medium. BALB/c blasts were generated by activation with $10 \mu\text{g ml}^{-1}$ LPS for 24 h. Blasts were depleted of T cells with anti-Thy-1 antibody (HK2.1) plus complement, washed, and irradiated (1,500 R). ECDI-treated blasts were fixed as described¹⁰. All cultures were incubated at 37 °C for 48 h, then 0.5–1 μCi [³H]thymidine was added. Cultures were collected after an additional 24 h incubation.

proliferation resulted when syngeneic lipopolysaccharide (LPS)-induced splenic blasts were added as accessory cells, even with submitogenic doses of anti-CD3. Similarly, addition of anti-CD28 produced vigorous proliferation at submitogenic doses of anti-CD3. Maximal proliferation was three- to fivefold higher than that obtained at the highest concentrations of anti-CD3 alone. Interleukin-2 (IL-2) secretion by CD4⁺ T cells was minimal when cultured on anti-CD3 alone, whereas the addition of anti-CD28 or accessory cells resulted in an increase in secretion commensurate with or exceeding the increase in the proliferative response (data not shown). Thus it seems that both accessory cells and anti-CD28 can provide the co-stimulatory signal required for proliferation at submitogenic doses of anti-CD3, or for optimal proliferation at saturating levels of TCR-mediated signals.

To determine whether co-stimulation could be due to increasing the strength of the TCR signal, we compared the effects of anti-CD28 and G7, a mitogenic antibody against Thy-1 which acts through the TCR pathway^{8,9}. Exposure of T cells to G7 and anti-CD3 results in a slight and additive increase in proliferation but, in contrast to anti-CD28, G7 has minimal effects at submitogenic doses of anti-CD3 (Fig. 1b). Thus, the co-stimulation with anti-CD28 seems to be distinct from the augmentation of proliferation by mitogens that operate through the T-cell antigen receptor pathway.

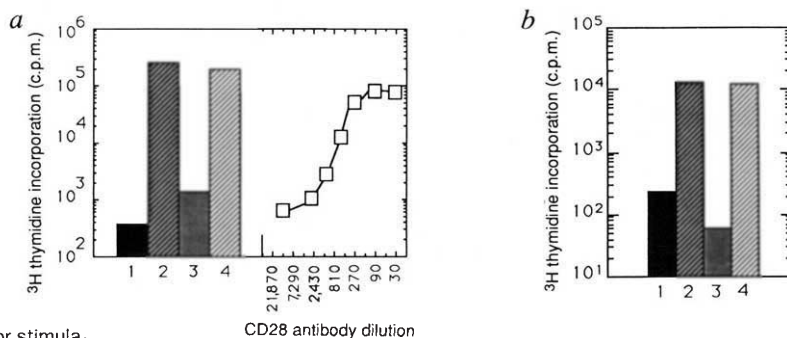
The similar effects of accessory cells and anti-CD28 suggests that anti-CD28 could be mimicking signals provided by a CD28 ligand on accessory cells. We therefore examined the effect of non-stimulatory Fab fragments of the anti-CD28 antibody on co-stimulation by accessory cells. Anti-CD28 Fab strongly

blocked co-stimulation by accessory cells, whereas Fab from a control hamster antibody did not (Fig. 1c). This result indicates that co-stimulation by accessory cells is mediated by interaction with CD28 on the T-cell surface.

IL-2-producing T-cell clones stimulated with chemically fixed antigen-presenting cells bearing the appropriate antigen and major histocompatibility complex (MHC) molecules fail to proliferate, but are instead induced into a state of anergy^{10,11}. Fixation of accessory cells with ECDI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) abrogates their capacity to co-stimulate freshly isolated CD4⁺ T cells (Fig. 1d). Similarly, the proliferative response of an I-A^d-restricted, λ bacteriophage repressor-specific Th1 (type-1 T helper) clone to antigen/MHC complexes was decreased by >100-fold by chemical fixation of antigen-pulsed cells (Fig. 2a). Addition of unfixed, unpulsed allogeneic accessory cells restored proliferation of the T cells in response to fixed, antigen-pulsed syngeneic cells, as in a similar system¹². Because the allogeneic spleen cells fail to stimulate the Th1 clones by themselves (data not shown), they must be providing a co-stimulatory signal that acts with TCR engagement. This signal was also provided by anti-CD28, which resulted in a dose-dependent restoration of T-cell proliferation (Fig. 2a).

To determine whether co-stimulation of Th1 clones by allogeneic spleen cells and CD28 antibody involves a common receptor, clones were stimulated with antigen-pulsed, ECDI-fixed syngeneic splenocytes and irradiated allogeneic splenocytes in the presence of anti-CD28 Fab (Fig. 2b). Th1 cell proliferation was reduced >100-fold by anti-CD28 Fab, but not control Fab. The anti-CD28 Fab was not toxic, as the

FIG. 2 A CD28-mediated interaction is necessary for antigen-specific proliferation of cloned T cells. *a*, Anti-CD28 provides a co-stimulatory signal for proliferation of cloned T cells. Cloned, λ repressor-specific Th1 cells (clone L2) were stimulated with: antigen only (lane 1); antigen and irradiated syngeneic spleen cells depleted of T cells (lane 2); antigen-pulsed, ECDI-fixed syngeneic spleen cells (lane 3); or antigen-pulsed, ECDI-fixed syngeneic spleen cells plus irradiated T-cell-depleted allogeneic spleen cells (lane 4). Right panel, L2 cells were stimulated with antigen-pulsed, ECDI-fixed syngeneic spleen cells and the indicated dilution of culture supernatants containing anti-CD28 monoclonal antibody. Proliferation of the stimulated T cells was determined after 2 days. Stimulation with anti-CD28 in the absence of antigen receptor stimulation failed to induce T-cell proliferation (data not shown). Similar results were obtained with a second cloned T-cell line, T32. *b*, Anti-CD28 Fab prevents co-stimulation of cloned T cells by allogeneic splenocytes. Cloned T cells (T32) were stimulated with antigen-pulsed, ECDI-fixed, irradiated splenocytes and either no additions (lane 1); irradiated allogeneic splenocytes (lane 2); irradiated allogeneic splenocytes and 100 $\mu\text{g ml}^{-1}$ anti-CD28 Fab (lane 3); and irradiated allogeneic splenocytes and 100 $\mu\text{g ml}^{-1}$ anti-CD28 and 20 U ml^{-1} recombinant IL-2 (lane 4). METHODS. L2 and T32 (gifts from M. Geffer and T. Briner) are cloned T-cell lines from a BALB/c mouse raised against λ repressor protein and respond to peptide 12–26 of λ repressor in the context of A^d by proliferation and secretion of IL-2 (M. Bix, personal communication). Cultures contained 2×10^4 L2 or T32 cells and, where indicated, 30 μM λ repressor peptide



(p12–26), 5×10^5 BALB/c (syngeneic) irradiated (3,000 R) T-depleted spleen cells, 5×10^5 antigen-pulsed, ECDI-fixed BALB/c T-depleted spleen cells, or 5×10^5 of antigen-pulsed, ECDI-fixed syngeneic spleen cells plus either antibody or 1.5×10^6 irradiated BALB.K (allogeneic) T-depleted spleen cells. For fixation, T-depleted spleen cells were pulsed with 30 μM p12–26 for 30 min at 37 °C, washed and fixed with ECDI as previously described¹⁰. The cells were extensively washed in serum-free medium and irradiated (3,000 R) before addition to cultures. The medium used for these experiments was RPMI-1640 supplemented with 10% FCS, 50 μM 2-mercaptoethanol and antibiotics. Cells were incubated for 48 h in flat-bottomed plates, pulsed with 0.5 μCi [³H]thymidine for 18 h, collected, and incorporated [³H]thymidine determined by scintillation counting. The standard errors of the mean of triplicate (a) or duplicate (b) cultures were all within 20% of the mean.

addition of IL-2 reversed the inhibition. These results indicate that co-stimulation of Th1 clones by spleen cells is mediated through the CD28 molecule.

To investigate whether CD28 co-stimulation also prevented the induction of anergy, T cells subjected to the various activation conditions were removed from stimuli, rested, and then tested for the ability to respond to antigen-pulsed, unfixed syngeneic spleen cells (Fig. 3). Cells initially exposed to spleen cells and antigen, or fixed spleen cells in the absence of antigen,

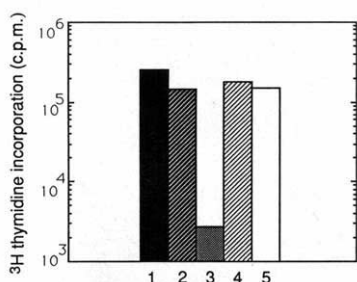


FIG. 3 CD28 signalling prevents the induction of T-cell anergy. L2 cells were precultured for 18 h under various conditions, re-isolated, rested for 3 days, and tested for responsiveness by restimulation with antigen and irradiated syngeneic spleen cells. The preincubation conditions were: antigen and irradiated syngeneic spleen cells (lane 1); ECDI-fixed, irradiated syngeneic spleen cells (lane 2); antigen-pulsed, ECDI-fixed, irradiated syngeneic spleen cells (lane 3); or antigen-pulsed, ECDI-fixed, irradiated spleen cells plus either allogeneic irradiated spleen cells (lane 4) or anti-CD28 antibody (lane 5). No significant proliferation was observed in any of the cultures in the absence of restimulation with antigen-pulsed syngeneic splenocytes. In parallel cultures anergized T cells proliferated in response to human rIL-2 equally well as non-anergized cells, indicating that the preincubation with ECDI-fixed antigen-pulsed cells did not affect T-cell viability.

METHODS. The preincubation cultures were in 24-well plates containing 5×10^5 cloned T cells and 30 μM p12–26 plus 5×10^6 irradiated syngeneic T-dependent spleen cells (lane 1); 5×10^6 ECDI-fixed syngeneic spleen cells (lane 2); 5×10^6 antigen-pulsed ECDI-fixed syngeneic spleen cells (lane 3); 5×10^6 antigen-pulsed ECDI-fixed syngeneic spleen cells plus 5×10^6 irradiated T-depleted BALB.K spleen cells (lane 4); or 5×10^6 antigen-pulsed ECDI-fixed syngeneic spleen cells plus a 1:10 dilution of culture supernatant containing anti-CD28 antibody (lane 5). After the preincubation cultures, the cloned T cells were reisolated by centrifugation on Ficoll–isopaque, washed, and rested without stimulation for 3 days at 37 °C. The rested T cells were restimulated as described (Fig. 2) except that duplicate cultures were used.

exhibited a vigorous response on restimulation. But the response of cells initially exposed to fixed spleen cells and antigen was >100-fold lower. The inclusion of unfixed allogeneic spleen cells or anti-CD28 in the primary cultures resulted in a vigorous response on rechallenge, demonstrating that co-stimulatory signals provided by the splenocytes or by anti-CD28 can prevent the induction of anergy in T cells.

We have shown here that CD28 can serve as a receptor for co-stimulatory signals that are required, in addition to TCR signals, for optimal induction of T cells and for preventing the induction of clonal anergy *in vitro*. It has been suggested that this requirement for two activation signals could have a role in the induction of peripheral tolerance *in vivo*^{1,2}. According to this model, T cells that encounter antigen on antigen-presenting cells that bear the ligand for the co-stimulatory receptor will become fully activated and carry out their usual role in the immune response. T cells that encounter antigen on cells that lack the co-stimulatory ligand would be rendered anergic. Because co-stimulatory activity is primarily restricted to haematopoietic cells, this could be a means of achieving functional tolerance to parenchymal cells that express antigens not expressed in the thymus. The interaction of CD28 with a ligand such as the B-cell activation antigen B7 (refs 13–17) could be important in this system. The availability of a monoclonal antibody against murine CD28 will allow us to address the relevance of the two-signal model to immune responses *in vivo*. □

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Homeodomain-independent activity of the *fushi tarazu* polypeptide in *Drosophila* embryos

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THE *Drosophila* segmentation gene *fushi tarazu* (*ftz*) encodes a homeodomain-containing protein, *ftz*, that can act as a DNA-binding activator of transcription¹⁻⁵. In the developing embryo, *ftz* is expressed in seven stripes⁶ which correspond to the even-numbered parasegments⁷. These parasegments are missing in *ftz*⁻ embryos⁸. When *ftz* is expressed throughout blastoderm embryos under the control of a heat-shock promoter, the odd-numbered parasegments are lost⁹. This 'anti-*ftz*' phenotype has been attributed to autoactivation of the endogenous *ftz* gene by the ectopically expressed protein¹⁰. Here we show that the same phenotype is induced by ectopic expression of a *ftz* polypeptide containing a deletion in the homeodomain. Thus, *ftz* can alter gene expression without binding directly to DNA.

To delineate functional domains in the *ftz* protein, we generated a series of *ftz*-deletion constructs which could be expressed

<i>a</i>	Transcriptional activator	Transcriptional activity	Anti- <i>ftz</i> phenotype
(1) <i>ftz</i> ³⁻⁴¹³		213	+++ (4/4)
(2) <i>ftz</i> ^{Δ274-302}		1	+++ (3/3)
(3) <i>ftz</i> ³⁻³¹³		14	+/- (1/2)
(4) <i>ftz</i> ^{3-171/223-337}		41	*
(5) <i>ftz</i> ¹⁷²⁻⁴¹³		167	- (3/3)
(6) <i>ftz</i> ²²³⁻⁴¹³		237	- (4/4)
(7) <i>ftz</i> ³⁻¹⁷²		1.5	- (2/2)
(8) <i>ftz</i> ²²³⁻³³⁷		1	- (2/2)

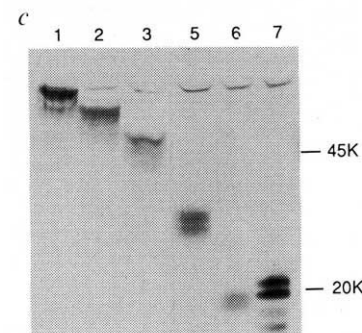
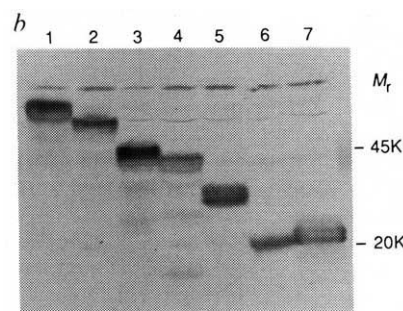


FIG. 1 *a*, Activities of *ftz* derivatives in tissue-culture cells and *Drosophila* embryos. To determine transcriptional activity of the *ftz* deletion derivatives, Schneider cells were co-transfected with the *ftz* expression constructs indicated and the *ftz*-dependent CAT reporter plasmid pD33NP6-CAT (ref. 1). Values given for transcriptional activity show CAT activities from cotransfected cell extracts relative to the CAT activity of extracts from cells transfected with pD33NP6-CAT alone. The same *ftz* derivatives were also ectopically expressed in *Drosophila* embryos and larval cuticles scored for an anti-*ftz* phenotype. The number of independent transformant lines obtained, as well as the number of transformant lines that produced the phenotype are indicated; +++ indicates the ability to induce a strong anti-*ftz* phenotype. Negative symbols indicate no phenotypic activity. Of the two *ftz*³⁻³¹³ transformant lines, one yielded very weak anti-*ftz* phenotypes and the other yielded only wild-type cuticles. *Transformants were not obtained with this construct. *b*, *c*, Immunodetection of *ftz* polypeptides from tissue-culture cells (*b*) and third instar larvae (*c*). Protein extracts isolated from Schneider cells transfected with *ftz* expression constructs or from heat-shocked third instar larvae of *ftz* transformant lines, were analysed by western blotting using an anti-*ftz* polyclonal antibody²⁰. Numbers above each lane correspond to the deletion constructs shown in *a*. The *ftz*²²³⁻³³⁷ polypeptide, which does not resolve on 10 or 12% polyacrylamide gels, was detected on higher percentage gels at about the same levels of expression (not shown). Crossreactive non-*ftz* polypeptides indicate the equivalence of sample loading.

METHODS. The expression constructs used for Schneider cell transfections and P-element-mediated transformations were derivatives of the plasmids pPac²¹ and pHT4²² respectively. During construction, some additional non-*ftz* amino acids were introduced at the termini of the *ftz* polypeptides. Deletions *ftz*³⁻⁴¹³, *ftz*^{Δ274-302}, *ftz*^{3-171/223-337}, and *ftz*³⁻¹⁷² encode MDPEFIKEEKLTMTRDP-(T)³*ftz* at its N terminus; *ftz*¹⁷²⁻⁴¹³ has MDPEFELGTRGSSR-(V)¹⁷¹*ftz* at the N terminus; *ftz*²²³⁻⁴¹³ and *ftz*²²³⁻³³⁷ encode MDPEFGACMPAGP-(V)²²³*ftz*. Non-*ftz* amino acids at the C termini are: for *ftz*^{3-171/223-337} and *ftz*²²³⁻³³⁷, *ftz*(E)³³⁷-GGILV; for *ftz*³⁻³¹³ the C terminus is *ftz*(K)³¹³-NLVYITCLCS. The non-*ftz* amino acids at the internal junction of *ftz*^{3-171/223-337} are *ftz*(V)¹⁷¹-PAGP-(V)²²³ *ftz*. Each of the *ftz* deletion derivatives expressed from the pPac and pHT4 vectors were identical, with the exception of *ftz*³⁻³¹³, in which the N-terminal amino acids are MRDP-(T)³*ftz* in pPac and MDPEFIKEEKLTMTRDP-(T)³ *ftz* in pHT4. The transfection protocol was essentially as described²¹. Transcriptional activity values

were normalized for protein concentration. Correcting for transfection efficiency by incorporating β -galactosidase values did not significantly alter the results. Corrections for variations in *ftz* polypeptide concentrations were not made as there was a variable loss of epitopes in each of the *ftz* derivatives. Embryos collected for heat shock from P-element-transformed²³ fly lines at 2.75–3.25 h after egg laying were washed onto screens and heat-shocked for 10 min in Eppendorf tubes submerged in a 36.5 °C water bath. Devitellinized larvae were mounted in Hoyer's mountant²⁴. Roughly 100 larvae from each transformant line were screened for cuticular phenotypes. Transformant lines with no anti-*ftz* activity were also phenotypically wild type after 15-min heat shocks. For western blots, protein extracts of Schneider cells transfected with 10 μ g *ftz*-expressing plasmid were prepared by repeated freeze-thaw cycles of transfected cells which were resuspended in 100 μ l 250 mM Tris, pH 8.0, 1.0 mM EDTA. Insoluble material was removed by centrifugation. Supernatants were electrophoresed in a 12% SDS-polyacrylamide gel. For protein extracts of transformant flies, third instar larvae were collected and heat-shocked at 36.5 °C for 1 h and homogenized in 2 $\times$ Laemmli sample buffer (5 μ l $\times$ buffer per mg larvae) 30-min after the heat shock. The equivalent of 2 mg wet weight larvae was electrophoresed in a 10% polyacrylamide gel. Following transfer to nitrocellulose, *ftz* polypeptides were detected using anti-*ftz* polyclonal antibodies²⁰ and alkaline phosphatase-coupled secondary antibodies.

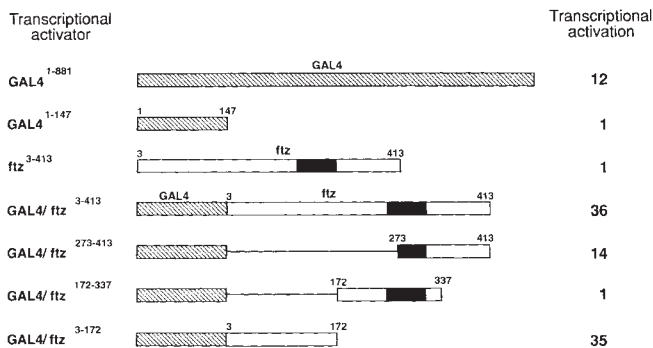


FIG. 2 Transcriptional activity of GAL4 and GAL4/ftz fusions assayed in *Drosophila* tissue culture cells. GAL4 and the GAL4/ftz chimaeric proteins

in cultured cells and developing embryos. The relative transcriptional activities of the deleted polypeptides were first measured in transfected cells (Fig. 1a). Plasmids expressing the various *ftz*-deletion constructs were cotransfected into *Drosophila* Schneider-2 cells together with a *ftz*-dependent chloramphenicol acetyltransferase (CAT), reporter plasmid. Each of the deletion derivatives was detected on a western blot (Fig. 1b). Not surprisingly, the relative CAT activities showed that the DNA-binding homeodomain was required but was not sufficient for activation of the *ftz*-dependent reporter gene (compare activities of *ftz*³⁻⁴¹³, *ftz*^{Δ274-302} and *ftz*²²³⁻³³⁷). Addition of N- or C-terminal sequences to the transcriptionally inactive homeodomain derivative restored transactivation potential. The presence of transactivation domains in the N and C termini of *ftz* was confirmed by fusing these domains to the DNA-binding domain of the yeast protein GAL4 and monitoring GAL4-dependent CAT reporter gene activity (Fig. 2). These data indicate that *ftz*, like other

were assayed for transcriptional activity in *Drosophila* Schneider cells by cotransfection of expression constructs with a GAL4-dependent CAT reporter. Values given for transcriptional activity show CAT activities from cotransfected cell extracts relative to the CAT activity of extracts of cells transfected with the CAT reporter construct alone.

METHODS. Transfections and CAT assays were as described for Fig. 1. Expression plasmids for GAL4 or GAL4/ftz fusions were constructed by inserting sequences encoding either GAL4¹⁻⁸⁸¹ or GAL4¹⁻¹⁴⁷ into a unique *Bam*HI site of a pPac derivative. The GAL4¹⁻¹⁴⁷ construct was used to make the GAL4/ftz fusions. The GAL4-dependent reporter was constructed by inserting a 600-base-pair *Sma*I/*Xho*I fragment containing the major GAL4 binding sites upstream of the GAL1 gene from pL1Δ21 (ref. 25) into the *Sal*I site of pD-33CAT. The amino acids encoded at the GAL4/ftz junctions were: GAL4(S)¹⁴⁷-PEFIKEEKLTRP-(T)³ *ftz* for GAL4/ftz³⁻⁴¹³ and GAL4/ftz³⁻¹⁷²; GAL4(S)¹⁴⁷-PEFK-(D)¹⁷² *ftz* for GAL4/ftz¹⁷²⁻³³⁷; GAL4(S)¹⁴⁷-PEFELGTRGSSRV-(E)²⁷³ *ftz* for GAL4/ftz²⁷³⁻⁴¹³. The C terminus encoded by the GAL4¹⁻¹⁴⁷ construct is GAL4(S)¹⁷⁴-PEF.

stimulatory transcription factors¹¹, contains modular DNA-binding and transcriptional-activation domains.

To determine the functional importance of these domains *in vivo*, we transformed heat-shock-inducible *ftz* deletion constructs into flies. Analysis by Southern (data not shown) and western blotting (Fig. 1c) confirmed that each of our transformants contained and expressed the correct constructs. Four (of four) full-length *ftz* transformant lines gave strong anti-*ftz* cuticular phenotypes characterized by the deletion of odd-numbered parasegments as previously described⁹ (Figs 1a, 3b). Surprisingly, the only deletion derivative that generated a strong phenotype was the *ftz* polypeptide lacking a functional homeodomain. Three (of three) *ftz*^{Δ274-302} transformant lines yielded cuticular phenotypes that were essentially identical to those generated by the full-length protein (Fig. 3c). Although many of the other *ftz* derivatives were transcriptionally active in cultured cells, all except *ftz*³⁻³¹³ showed no activity in this assay

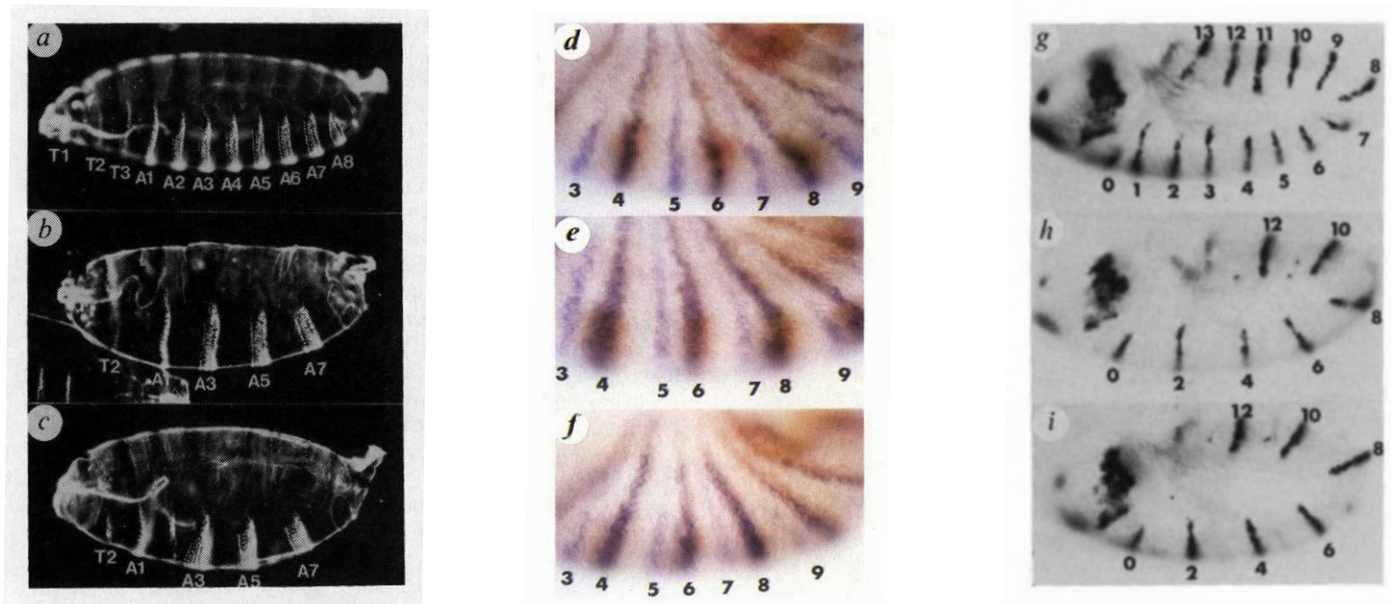


FIG. 3 Cuticular phenotypes and patterns of *en* and *wg* expression are similar in heat-shocked *ftz*³⁻⁴¹³ and *ftz*^{Δ274-302} embryos. Cuticular phenotypes (a-c), *ftz* and *en* expression (d-f), and *wg* expression (g-i) patterns are compared in heat-shocked wild type (a, d, g), *ftz*³⁻⁴¹³ (b, e, h) and *ftz*^{Δ274-302} (c, f, i) embryos. a-c, Dark-field photomicrographs for first instar larval cuticles. The wild-type cuticle (a) has been photographically reduced by ~30% relative to those in b and c. Photomicrographs d-f show embryos double-stained for *en* transcripts (blue) and *ftz* protein (brown) 45-min after heat shock. At this stage (3.5-4 h after egg laying), *ftz* stripes have normally resolved to stripes one or two cells wide which overlap

completely with the even-numbered stripes of *en* (d). The anterior margins of *ftz* stripes in e and f are shifted anteriorly and coincide with the anterior margins of the widened *en* stripes. g-i, Embryos stained for *wg* transcripts 100 min after heat shock. Odd-numbered *wg* stripes were repressed in both *ftz*³⁻⁴¹³ (h) and *ftz*^{Δ274-302} (i) embryos.

METHODS. Cuticles were prepared as described in Fig. 1 legend. Whole-mount *in situ* hybridization followed ref. 26; double staining used antibody staining first²⁷, followed by *in situ* hybridization (A. Manoukian and H.M.K., unpublished).

(Fig. 1a). Thus, the ability to induce a strong anti-*ftz* phenotype required the regions of *ftz* encompassing both activation domains but did not require a functional homeodomain.

Ish-Horowicz *et al.*¹⁰ have suggested that transient activation of the endogenous *ftz* gene by ectopic *ftz* leads to an anterior widening of the endogenous *ftz* stripes. This, in turn widens the even-numbered stripes of the segment polarity gene *engrailed* (*en*) and represses the odd-numbered stripes of the segment polarity gene *wingless* (*wg*). We tested whether our homeodomain-deleted *ftz* derivative also caused these changes (Fig. 3). Double-staining of *ftz* protein and *en* transcripts shows that the even-numbered *en* stripes were widened by both the full-length and homeodomain-deleted proteins, and that the anterior margins of the widened *en* stripes corresponded to the anterior margins of the widened *ftz* stripes (Fig. 3e,f). Both *ftz* derivatives also caused the repression of odd-numbered *wg* stripes (Fig. 3h,i). Thus, although the *ftz* homeodomain is required for DNA binding (H.M.K., unpublished results) and to rescue a *ftz* null phenotype (M. Mueller and W.J. Gehring, personal communication), it was not necessary to generate the anti-*ftz* phenotype. This result contrasts with previous ectopic expression studies in which the homeodomains encoded by the genes *Ultrabithorax*, *Antennapedia* and *Deformed* were shown to be crucial for the induction and determination of cuticular phenotypes¹²⁻¹⁴.

The anti-*ftz* phenotype that we observe is probably initiated by protein-protein interactions alone. Such interactions may recruit ectopically expressed *ftz* into a protein-DNA complex that results in the activation of the endogenous *ftz* gene anterior to its normal domain of expression. Alternatively, ectopically expressed *ftz* might sequester or inactivate a factor that is a negative regulator of the *ftz* gene. There have been several reports of transcripts encoded by homeobox-containing genes in *Drosophila*¹⁵, *Xenopus*^{16,17} and mice¹⁸ which are spliced so that their homeodomains are deleted or out of frame. In addition, a targeted deletion of the homeobox in the murine *En-2* gene was not lethal in homozygous *En-2^{hd}/En-2^{hd}* mice¹⁹. Our results suggest that the proteins encoded by these genes could function in the absence of their DNA-binding homeodomains by means of protein-protein interactions. □

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Bipotential precursors of B cells and macrophages in murine fetal liver

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LYMPHOCYTES (B and T cells) derive continuously from the same multipotential stem cells that produce myeloid cells, including erythrocytes, granulocytes and macrophages^{1,2}. Tri- and bipotential myeloid intermediates between the multipotential stem cells and later unipotential cells have been identified using clonal methods in culture. Although similar methods have detected committed pre-B cells in mouse fetal liver³, earlier progenitors with additional non-B lineage options have not been demonstrated in normal tissues. We report the characterization and purification of fetal liver cells that generate clones containing both macrophages and B cells, identified biochemically and morphologically. The common origin of the two cell types was shown by culture of single precursor cells. Their dual potential and unrearranged immunoglobulin loci place the precursors before exclusive B-lineage commitment in the haematopoietic hierarchy. The availability of such cells in purified form will allow direct study of lineage choice in cells having both lymphoid and non-lymphoid options.

Haematopoietic precursors appear in mouse fetal liver at day 11 of gestation but mature B cells do not appear until day 17 (refs 4, 5). We have shown that 12-day fetal liver cells cultured with γ -irradiated marrow fibroblasts and interleukin 7 can generate clones of B cells that can be induced to differentiate terminally into immunoglobulin-secreting cells with appropriate B-cell mitogens⁶⁻⁸. The clonal precursors have unrearranged immunoglobulin loci and belong to the subpopulation, recognized by monoclonal antibody AA4.1 (ref. 9), that includes multipotential hematopoietic stem cells¹⁰. It therefore seemed possible that these early B-cell precursors might also be able to differentiate into other lineages.

In the present study, we purified the precursors extensively to allow examination of the clonal progeny of single isolated cells. Cells were successively panned on antibody-coated plates to separate them according to surface markers of stem cells (AA4.1 and Ly6A^{11,12}), pre-B cells (B220) and macrophages (Mac-1). Cells retained on antibodies to B220 and Mac-1 (fraction IIa, Table 1) yielded clones in culture which contained either macrophages or B cells but not both. In contrast, limiting dilution cultures of the non-retained (B220⁻Mac⁻, fraction IIb) cells yielded culture wells containing B cells together with macrophage-like cells. When this fraction was further panned on plates coated with antibodies to Ly6A, the retained fraction (IIb, Ly6A⁺) contained over 50% of the total B-cell precursors in the original unfractionated fetal liver and only 0.1% of the starting cells.

The rest of the experiments focused on the AA4.1⁺B220⁻Mac⁻Ly6A⁺ (fraction IIb) population. The frequency of B-cell precursors was 1:3 to 1:7 in limiting dilution cultures with IL-7 and γ -irradiated S17 cells. Surprisingly, practically all of the wells containing small, round growing B-cell precursors also contained growing, plastic-adherent macrophage-like cells (Fig. 1). The frequency of mixed clones was independent of numbers of cells seeded down to 0.1 cells on average per well, strongly suggesting clonal origin of the mixed cultures. The high frequency of dual precursors made it possible to test rigorously for single cell origin by micromanipulation of single cells into culture wells under direct microscopic visualization. Of 70 single cells cultured alone, 13 formed dual B-lineage/adherent cell clones, and no wells contained B-lineage cells alone. These

TABLE 1 Fractionation of B-cell and macrophage precursors in fetal liver

Fraction	AA4.1	Antibodies		Ly6A	Per cent total fetal liver	Number of clones per fetal liver (frequency)			
		B220	Mac-1			Containing B	B only	M only	Mixed B and M
Unfractionated					100	1,620 (1:716)	ND	ND	
I	+				1.6	1,562 (1:12)	ND	ND	
IIa*	+	+	+		0.5		496 (1:10)	1,933 (1:3)	<95 (<1:60)
IIb	+	—	—		1.4		507 (1:32)	4,060 (1:4)	812 (1:20)
IIIa	+	—	—	—	1.0		322 (1:36)	2,900 (1:4)	<190 (<1:60)
IIIb	+	—	—	+	0.1		<20 (<1:60)	657 (1:3)	986 (1:3)†

Liver cells (typically 2×10^7) from day -12 C57BL/6 mouse fetuses were separated by panning on plastic petri plates coated with the indicated antibodies⁸. Cells adhering to AA4.1 (Fraction I) were separated on combined anti-B220 (14.8; ref. 24) and anti-Mac-1 (M1/70; ref. 25) to yield adherent (fraction IIa) and non-adherent (fraction IIb) cells. Fraction IIb was panned again on anti-Ly6A to yield Fractions IIIa and IIIb. Panning enriched the desired phenotype typically by 80-fold, determined by immunofluorescent straining. Fractionated cells were cultured at limiting dilution over adherent layers of γ -irradiated (20 Gy) S17 fibroblasts (500–2,000 per well) in 200 μ l OptiMEM (GIBCO) containing 10% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol and 100 U ml⁻¹ recombinant IL-7. After 10 d, macrophages and B cells were identified by morphology (Fig. 1). B-cell identity was routinely confirmed by assay of secreted immunoglobulin after further incubation of non-adherent cells over irradiated S17 cells with IL-7, to a total of 12–15 d, followed by 8 d stimulation over irradiated S17 cells with 25 μ g ml⁻¹ lipopolysaccharide. Results are representative of 10 similar separations. Plus sign denotes adhesion to an antibody and a minus sign, non-adhesion; ND, not determined; B, B cell; M, macrophage.

* Fraction IIa included cells expressing either B220 or Mac-1, or both.

† Frequencies of bilineage cultures significantly exceeded those predicted by coincidence of unipotential macrophage- and B-cell precursors ($p < 0.01$, Fisher's exact test) in limiting dilution microcultures containing an average of 0.3, 0.2 or 0.1 cells per well.

experiments conclusively established the single cell origin of the B-lineage/adherent cell clones.

Five B-lineage/adherent cell clones from micromanipulated precursors were further analysed for their capacity to differentiate. Non-adherent B-lineage cells were subcultured with IL-7 and S17 cells, expanded to 2×10^5 – 5×10^6 cells and stimulated with bacterial lipopolysaccharide in the presence of S17 cells¹³. Immunoglobulin concentrations were measured in the supernatant using a monoclonal antibody¹⁴; the five clones yielded IgM at 170, 250, 260, 560 and 2,900 ng ml⁻¹ (ELISA sensitivity 5 ng ml⁻¹) but none was detected in stromal cell controls including macrophage subclones. The adherent cells from the same mixed clones were similarly expanded by culture with macrophage colony-stimulating factor (M-CSF). The cells retained a macrophage-like morphology, expressed the macrophage-specific enzyme naphthol AS-D chloroacetate esterase (Fig. 1) and did not respond to lipopolysaccharide by proliferation and immunoglobulin secretion.

The identity of the subclones was also tested by assessing expression of B-cell- and macrophage-specific mRNAs. Total complementary DNA was prepared and amplified from the expanded clones using a recently described technique providing representative amplification from small numbers of cells¹⁵. As shown in Fig. 2, subclones expanded with IL-7 expressed abundant mRNA for B-cell markers *C μ* (immunoglobulin heavy chain) and *Rag2* (recombination activating gene 2; ref. 16), but no detectable message for macrophage markers lysozyme or *c-fms* (the M-CSF receptor). In contrast, the corresponding adherent populations expressed high levels of lysozyme and *c-fms* but no detectable *C μ* or *Rag2* (Fig. 2a). The levels of expression were comparable to unipotent pre-B cells and macrophages expanded with IL-7 and CSF-1, respectively.

Figure 2b also shows hybridization of the same probes to amplified cDNA from S17 fibroblast cells or from fetal liver cells not retained by antibody AA4.1 (99% of fetal liver cells). Both cell types were positive for ribosomal gene expression¹⁷, which we used as a control, but hybridized only weakly with the B-lineage- or macrophage-specific probes. Strong expression of the B-cell and macrophage markers was confined to AA4.1⁺ populations, consistent with the nearly complete segregation of macrophage and B-cell precursors into these fractions. Hybridization of the Ly6A probe was, as expected, 20-fold stronger in the subfraction of cells binding to anti-Ly6A antibodies. These results collectively confirmed the specificity of the amplified cDNA technique and the efficiency of the cell separation.

We determined the configuration of the immunoglobulin gene loci in B-cell and macrophage subclones derived from bipoten-

tial progenitors. The polymerase chain reaction (PCR) was used to probe for a genomic fragment¹⁸ diagnostic of germ-line configuration in the immunoglobulin heavy chain locus. As shown in Fig. 3, this *J_H1* fragment was retained in the macrophage DNA from clone 4 but partially lost in the B-lineage DNA from the same clone. DNA from S17 fibroblasts provided a diploid standard for amount of the *J_H1* fragment and a fragment of the platelet-derived growth factor-receptor gene served as an additional diploid standard for all cell types. A twofold difference in the amount of the germ-line fragment could be readily discerned and the macrophages have a diploid

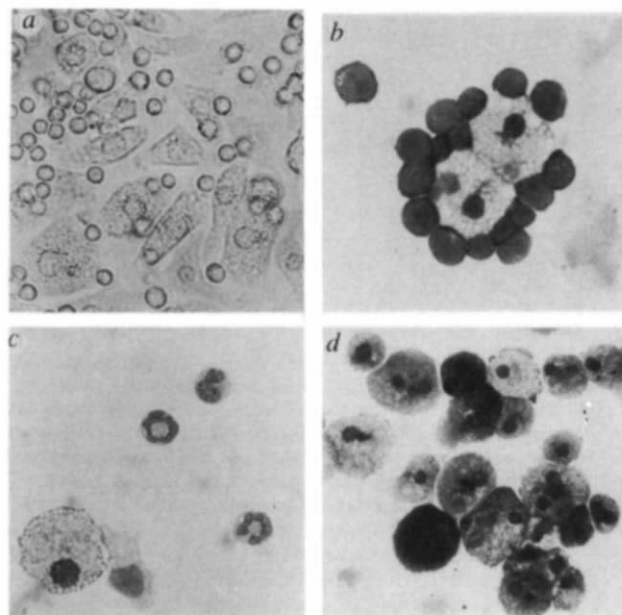


FIG. 1 Cells derived from single micromanipulated fraction IIIb fetal liver cells (see Table 1). a, Macrophage-B-lineage clone by phase contrast microscopy after 8 d culture. Adherent macrophages have displaced the S17 fibroblasts from the field of view. b, B-cell-macrophage clone and c, Neutrophil-macrophage clone cytopsin preparations stained with May-Grunwald-Giemsa. The clumping of cells in b is a preparative artefact and was not evident in culture or in suspensions before drying. d, Cytopsin preparation of adherent cells derived from a B-cell-macrophage clone and expanded in medium containing 80 ng ml⁻¹ recombinant human M-CSF. Cytoplasmic granules are stained positively for naphthol AS-D chloroacetate esterase (Sigma Diagnostics).

complement of J_H1 , establishing that both chromosomes were in germ-line configuration (Fig. 3). Thus immunoglobulin heavy chain loci must have been unrearranged in the precursors of the bilineage clones and rearrangement began only in progeny committed exclusively to B-lymphocyte differentiation.

The nature of the bipotential precursors was further explored by examining their cytokine dependence and potential to differentiate into additional hematopoietic lineages. Purified cells required S17 cells and IL-7; macrophage progeny probably required S17 cells to provide factors such as M-CSF. Neither interleukin-3 (IL-3), *c-kit* ligand (KL; ref. 19), IL-1, M-CSF nor combinations of them could substitute for S17 cells. When IL-3, KL and erythropoietin were included with S17 cells, more cells with myeloid potential were detected but the frequency of bilineage B-cell/macrophage clones was unaffected. Culture of 35 single micromanipulated cells in these conditions yielded 6 B-cell macrophage clones, 14 macrophage only, 4 multilineage erythroid-megakaryocyte-granulocyte and 1 neutrophil-macrophage clone. Of over 100 clones expanded under these

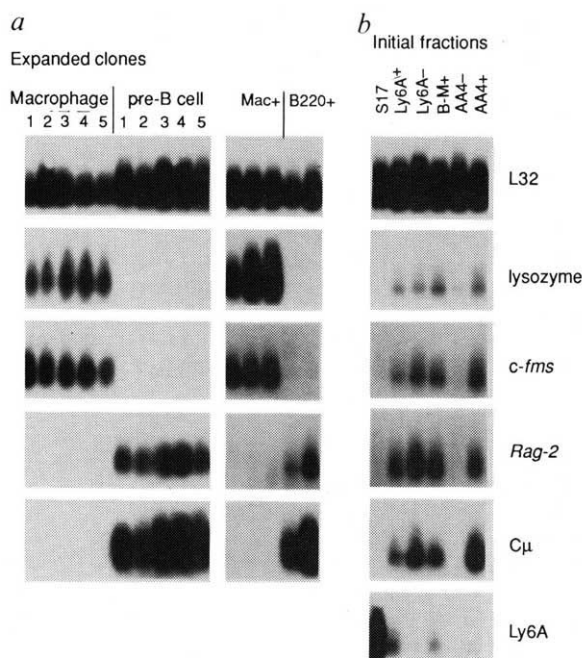


FIG. 2 Lineage-specific gene expression in fractionated fetal liver cells and macrophage and B-cell subclones. *a*, Gene expression in cells cultured from fetal liver. The adherent macrophages and non-adherent B-cell precursor components of each of 5 macrophage-B-lineage clones were expanded separately (see Table 1 legend and Fig. 1). Amplified poly(A)⁺ cDNA was electrophoresed, blotted and hybridized to the indicated probes. Control cDNAs were similarly amplified from 3 independently obtained populations of panned Mac-1-positive cells expanded in M-CSF and from 2 preparations of panned B220-positive cells expanded with IL-7. *b*, Gene expression in fresh fetal liver cell fractions. Southern blots of amplified poly(A)⁺ cDNA were hybridized to the indicated probes. Fractionation by panning as detailed in Table 1 yielded samples: AA4⁻, AA4⁺ (fraction I), B-M⁺ (fraction IIa), Ly6A⁻ (IIIa) and Ly6A⁺ (IIIb). Amplified cDNA from S17 cells was also analysed as a control.

METHODS. To prepare amplified poly(A)⁺ cDNA, 10^3 – 5×10^4 cells were lysed in 50 μ l buffer (5M guanidine isothiocyanate, 20 mM 1,4-dithioerythritol, 25 mM sodium citrate, pH 7.0, 0.5% Sarkosyl). After addition of 25 μ l 7.5 M ammonium acetate containing 20 μ g glycogen as carrier, nucleic acids were precipitated with 2 volumes of 95% ethanol, washed twice with 70% ethanol, dried and resuspended in 15 μ l water containing Inhibit Ace (5'-3', Inc). Part of each sample (1 μ l) was used to prepare amplified cDNA representing all poly(A)⁺ mRNA¹⁵. One tenth of the amplified material was separated on a 1.5% agarose gel and blotted onto nylon membranes. Filters were hybridized with 3'-specific probes for L32 ribosomal protein¹⁷, mouse lysozyme²⁶, *c-fms*²⁷, *Rag-2*, (ref. 16), Ly6A (ref. 28) and C μ (ref. 29). After washing the filters with 0.1 $\times$ SSC, 0.1% SDS at 70 °C, autoradiograms were exposed for 2 h (L32 and lysozyme), 24 h (C μ) or 72 h (*c-fms*, *Rag-2* and Ly6A).

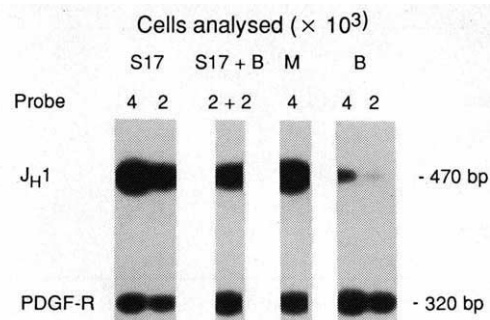


FIG. 3 Configuration of the immunoglobulin gene loci in B-cell and macrophage subclones from a single micromanipulated precursor cell. DNA samples from S17 cells and from the B-cell (B) and macrophage (M) components of clone 4 were amplified using a 3' primer within the J_H1 gene and a 5' upstream primer to yield a 473-base-pair fragment from germ-line DNA¹⁶. A second primer pair was used to amplify 320 base pairs within the PDGF B-chain receptor gene. PCR was carried out for 22 cycles on samples of 4×10^3 or 2×10^3 cells as indicated. The mixture of DNA from 2×10^3 S17 cells and 2×10^3 B-lineage cells is a control to show that hybridization intensity depended on amount of specific sequence rather than amount of total DNA. Filters were hybridized with a J_H1 probe (from the plasmid GW46 containing the *Bam*HI genomic 1-kilobase fragment, digested with *Bam*HI–*Hind*III) and a PDGF-B receptor probe³⁰. Autoradiograms were exposed for 24 h; identical results were obtained for 5 independent clones.

conditions, only B-cell-macrophage clones contained identifiable B-lineage cells. T-lymphocyte potential was not examined.

To test the relationship to the multipotential cells capable of long-term multilineage reconstitution *in vivo*, we assayed the bipotential precursor fraction for ability to sustain production of erythroid cells for 6 months in irradiated mice²⁰. Although this fraction contained most of the bipotential precursors present in fetal liver, it was practically devoid of reconstituting activity *in vivo*, having <5% of the reconstituting activity of unfractionated fetal liver that was recoverable in other fractions. Thus, the macrophage-B-lineage precursors are distinct from multipotential stem cells and do not have additional myeloid potential.

Our observations establish conclusively the ability of normal murine haematopoietic precursors to generate clones *in vitro* containing both lymphoid and myeloid cells. About half of all B-cell precursors in day-12 fetal livers were found to have dual potential. The existence of such precursors in adult bone marrow²¹ has been suggested on the basis of growth of both lineages in microcultures of limiting numbers of cells, but at low frequencies, in experiments in which comparable numbers of cultures could have been initiated with more than one cell. Immortalized B-cell lines with CD5 surface expression have also been reported to generate macrophage-like cells^{22,23}. However, our result demonstrates derivation of the two lineages from untransformed cells that have not yet rearranged their immunoglobulin loci, whereas the macrophage-like cells obtained from B-cell lines share the characteristic rearrangements of those lines. The significance of our observations to monocyte production *in vivo*, the possible existence of bipotential precursors in adult marrow and their relationship to the hematopoietic precursors which commit initially to T-lymphocyte differentiation, remains unclear but accessible to study. □

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***Fusarium solani* cutinase is a lipolytic enzyme with a catalytic serine accessible to solvent**

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LIPASES belong to a class of esterases whose activity on triglycerides is greatly enhanced at lipid-water interfaces¹. This phenomenon, called interfacial activation², has a structural explanation: a hydrophobic lid, which at rest covers the catalytic site, is displaced on substrate or inhibitor binding, and probably interacts with the lipid matrix³⁻⁶. *Fusarium solani pisi* cutinase belongs to a group of homologous enzymes of relative molecular mass 22-25K (ref. 7) capable of degrading cutin, the insoluble lipid-polyester matrix covering the surface of plants⁷, and hydrolysing triglycerides^{7,8}. Cutinases differ from classical lipases in that they do not exhibit interfacial activation; they are active on soluble as well as on emulsified triglycerides. Cutinases therefore establish a bridge between esterases and lipases. We report here the three-dimensional structure of a recombinant cutinase from *F. solani pisi*, expressed in *Escherichia coli*^{9,10}. Cutinase is an α - β protein; the active site is composed of the triad Ser 120, His 188 and Asp 175. Unlike other lipases, the catalytic serine is not buried under surface loops, but is accessible to solvent. This could explain why cutinase does not display interfacial activation.

Crystals of recombinant cutinase from *F. solani pisi* were obtained by the vapour diffusion technique using polyethylene-glycol PEG 6000 as a precipitant¹⁰. The structure was solved at 3.0 Å by the multiple isomorphous replacement method using four mutant cutinase derivatives (see Table 1). The resolution was extended to 1.6 Å and the model refined to a *R*-factor of

0.154 (Table 1). No density could be observed for 16 amino acids at the N terminus and two at the C terminus. Amino acids 1 to 15 are part of a propeptide, not present in the natural enzyme, which could explain their observed disorder. Nevertheless, cutinase protein is not expressed properly without these amino acids. The present model is therefore composed of 196, of the 199 amino acids of the native enzyme, and 190 water molecules.

Cutinase is a compact one-domain molecule, $\sim 45 \times 30 \times 30$ Å³ (Fig. 1a), which is smaller than the three other structurally characterized lipases^{3,4,5,6}. It is an α - β protein, with a central β -sheet of five parallel strands covered by five helices on either side of the sheet (Fig. 1a). A sixth strand does not belong to the central sheet. Loops are generally short compared to those from the *Rhizomucor meihei* lipase³ (RML), except loops 24-32, 75-90, 157-166 and 173-189. RML³, human pancreatic lipase⁵ (HPL), *Geotrichum candidum* lipase⁶, carboxypeptidase-II¹¹, *Torpedo californica* acetylcholinesterase¹² and haloalkane dehalogenase¹³, all show similarities in their central β -sheets^{6,12}. The central β -sheet of cutinase superimposes well with strands 3 to 7 of the central sheet from RML and HPL. However, loops and helices connecting these strands are different.

The active site of cutinase is composed of the catalytic triad Ser 120, His 188 and Asp 175 (Fig. 2). The triad serine and aspartic acid were chemically identified as amino acids 120 and 83, respectively¹⁴. Amino acid 188 is the only histidine in the

TABLE 1 Crystallographic data of *F. solani pisi* cutinase

	Native	Ser 4 Cys	Ser 92 Cys	Ser 120 Cys	Ser 129 Cys
No. of measurements*	114,297	15,562	14,351	15,541	15,420
No. of unique reflections	39,563	4,451	4,457	4,592	4,683
Completion (%)	83	91	91	98	97
Resolution limit (Å)	1.25	2.7	2.7	2.7	2.6
<i>R</i> -sym (%)†	6.1	5.3	5.2	4	4.1
<i>R</i> -iso (%)‡		24	11.1	14	23.5
<i>R</i> -Fhle§		0.58	0.58	0.56	0.49
Phasing power		1.3	1.5	1.9	2.4
No. of sites		1	2	1	1
Relative occupancy		4.9	2.7/2.0	3.2	5.9

Mean figure of merit 0.77 for 2,869 reflections in the range 6.0-3.0 Å.

Structure was solved from monoclinic crystals (P₂), with cell dimensions 35.12, 67.3, 37.05 Å and $\beta = 93.9^\circ$ (ref. 10). These crystals contain 38% solvent. The 14 possible Ser to Cys single mutants were prepared, from which four (Ser 4 Cys, Ser 92 Cys, Ser 120 Cys, Ser 129 Cys) were crystallized isomorphously to the native enzyme, and led to useful derivatives when soaked or co-crystallized with Hg(CH₃COO)₂. Native and derivative data sets were collected on a Siemens X100A multiwire detector placed on a Rigaku RU200 rotating anode using a copper target. Data were reduced using the XDS^{20,21} or the Xengen²² software packages. The structure was solved at 3.0 Å by multiple isomorphous replacement (MIR) using these derivatives with the CCP4 Program Suite, Daresbury Laboratory (SERC). The MIR map was of sufficient quality to place all- α β -strands and helices in recognizable densities, using TURBO-FRODO²³. The model was then completed (180 amino acids) and the side chains placed in density, according to sequence information^{7,9} and using the positions of the cysteine derivatives as starting points. The model was then subjected to one cycle of simulated annealing (2.5-ps of heating) with XPLOR²⁴, using MIR phases as restraints. The *R*-factor dropped from 0.52 to 0.27, and was accompanied by a substantial improvement of the stereochemistry of the model. Refinement was pursued with calculated phases, and the resolution was extended to 2.7, 2.2, 2.0, 1.7 and 1.6 Å, respectively. Some minor errors were corrected manually, and 16 residues added. Our sequence numbering is based on the recombinant sequence⁹. Numbering of the native enzyme⁷ is obtained by subtracting 15 to our numbering. The *R*-factor is 0.154 in the 6.0-1.6-Å resolution range, for 18,867 reflections with $I > 1\sigma(I)$. Some preliminary refinement has been performed at 1.25 Å (*R* = 0.171), but the present model description is based on the 1.6 Å model. The r.m.s. deviations for bond lengths and angles are 0.023 Å and 2.23°, respectively. Average B-factors for main chain, side chains and water molecules are 11.1 Å², 13.6 Å² and 39.8 Å², respectively.

* Data integration using XDS (native) and Xengen (derivatives).

† *R*-sym = $\sum |I - \langle I \rangle| / \sum \langle I \rangle$.

‡ *R*-iso = $\sum |I_{\text{deriv}} - I_{\text{native}}| / \sum I_{\text{native}}$.

§ Phasing power = $r.m.s. F_h/E$, F_h , heavy-atom structure factor; E , residual lack of closure.

|| *R*-Fhle = $S|F_{h(\text{obs})} - F_{h(\text{calc})}| / \sum F_{h(\text{obs})}$.

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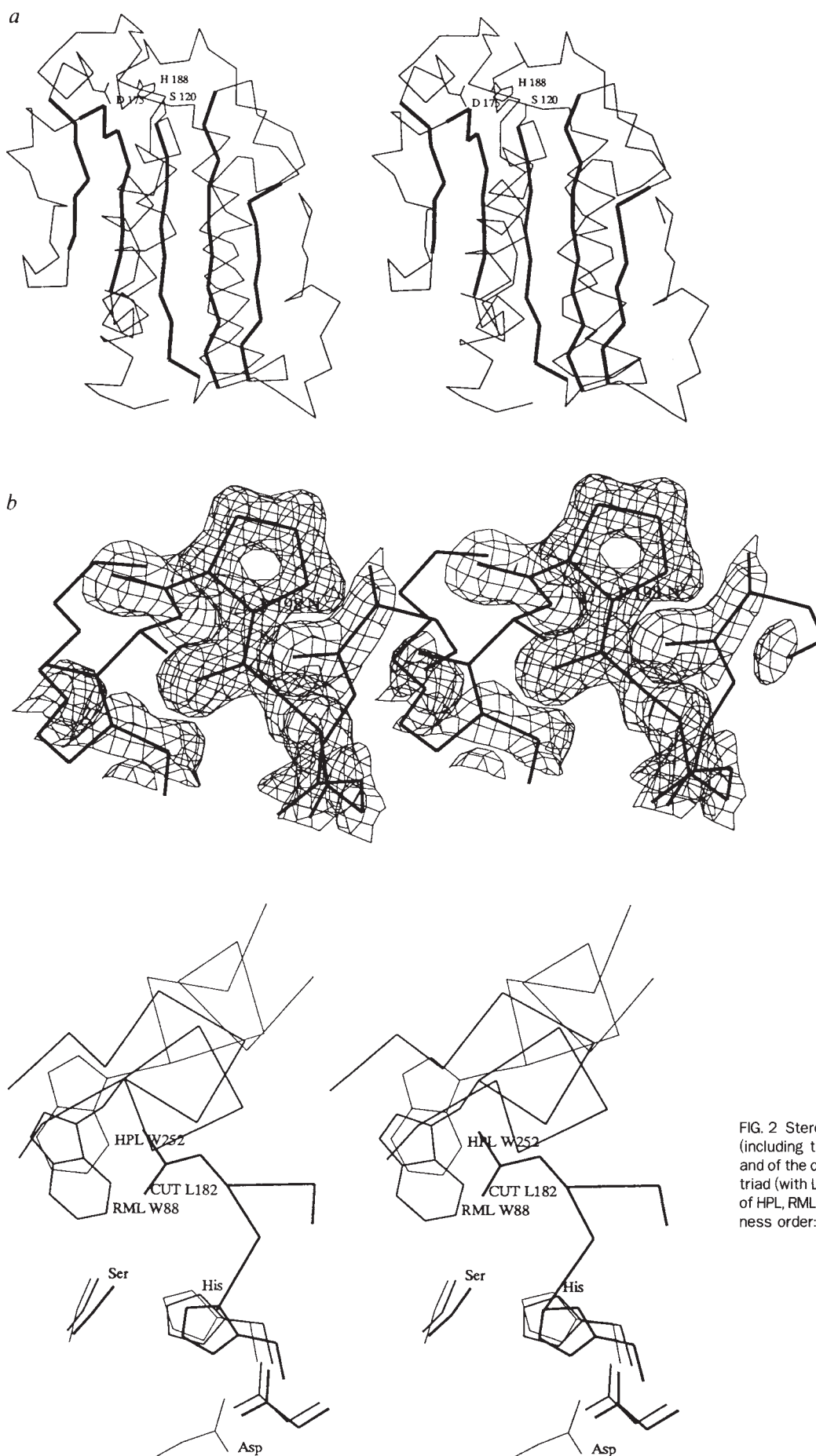
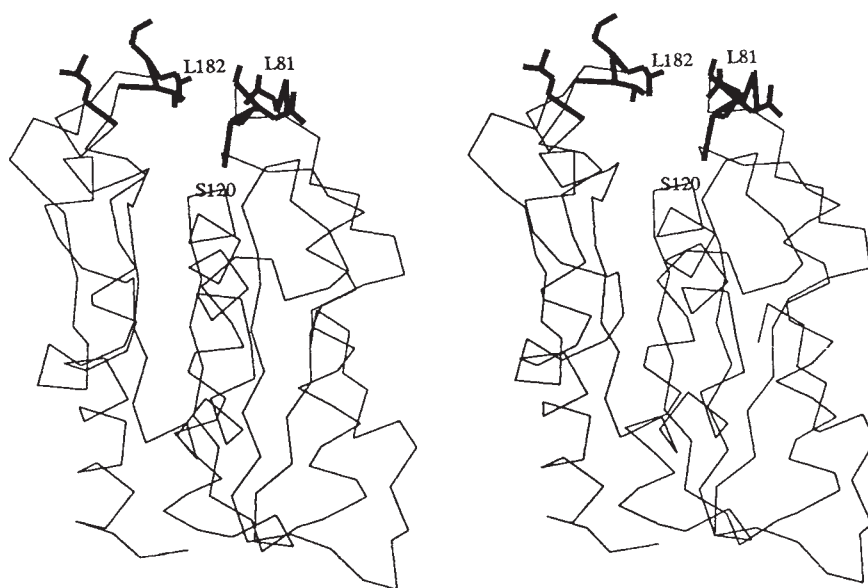


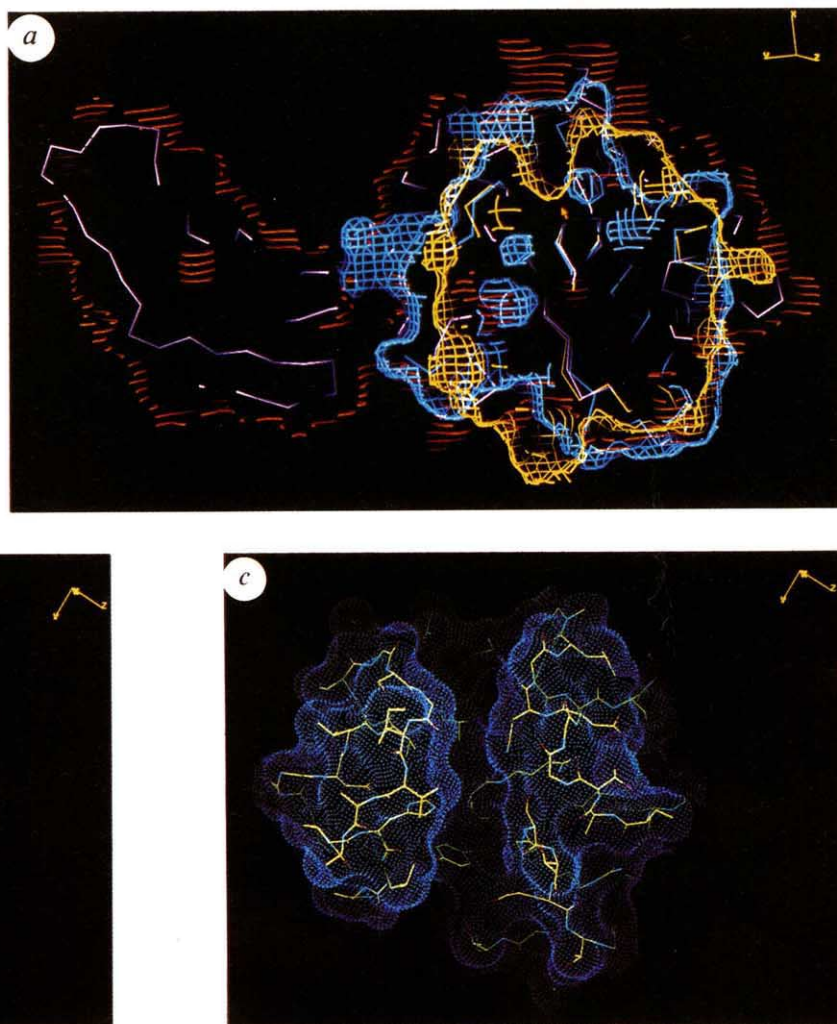
FIG. 3 Stereo view of the $C\alpha$ tracing of cutinase, with the side chains of amino acids belonging to the hydrophobic area, above the catalytic serine 120, traced in thick lines (amino acids Leu 81, Gly 82, Ala 85, Leu 86, Pro 87, Leu 176, Leu 182, Ileu 183, Val 184; for clarity, only residues 81 and 182 are numbered).



sequence¹⁴. The chemical assignment of the triad aspartic acid could seem suspect owing to the lack of conservation of aspartic acid 83 (among three cutinases sequences) compared to the invariant aspartic acid 175¹⁴. Moreover, the sequence order of the triad amino acids, Ser < Asp < His corresponds to that seen in other lipases³⁻⁶. The active site stretch of amino acids containing serine 120 (Gly-Tyr-Ser-Gln-Gly) matches the con-

sensus sequence (Gly-His/Tyr-Ser-X-Gly) commonly present in lipases^{3,5}. Ser 120 is situated in a β -hairpin, which can be classified as type II' (refs 15, 16), between strand 112-119 and helix 121-132. This serine has the ϵ conformation ($\phi = 60^\circ$ and $\psi = -123^\circ$), a feature common for the second amino acid of these β -hairpins, close to the stable region of conformational space around $\phi = 60^\circ$ and $\psi = -60^\circ$ (classified as E; ref. 17). All

FIG. 4 *a*, Colour view of the solvent-accessible surfaces (program MS from Connolly²⁵ and SPLINE from Colloc'h and Mornon²⁶) of cutinase (yellow), RML (blue) and HPL (red). Proteins are within the coloured contours. A slice containing the active-site serines (red arrow) is selected across the proteins. Left, C-terminal domain of HPL protrudes out of the common core of the three proteins made from their N-terminal domains. *b*, Colour view of the solvent-accessible surfaces of the crystallographic native cutinase structure. Only the area above the active-site crevice is shown. Bridges formed by residues Leu 81 and Leu 182 are visible over the active-site crevice. *c*, Colour view of the solvent-accessible surfaces of the double mutant Leu 182 Gly and Leu 81 Gly obtained by modelling. Only the area above the active-site crevice is shown. Bridges observed in *b* are absent.



these characteristics are found in the active site of other lipases¹⁵. After superposition of the corresponding parts of the central sheets of RML, HPL and cutinase, the catalytic triads almost superpose, especially the atoms involved in the catalytic process (O γ , N δ 1, O δ 1; Fig. 2). Their serines and histidines come from topologically homologous elements, but the aspartic acid of HPL (Asp 176) has a different topological origin from those from RML (Asp 203) and cutinase (Asp 175; Fig. 2). Cutinase triad also superposes with the catalytic triad of bovine trypsin, as do the three known lipases^{3,5,6}.

The short helices of HPL (247–254) and RML (85–92) constitute the central parts of the flaps. They contain tryptophan amino acids (252 and 88, respectively) that superpose well and cover the catalytic serine (Fig. 2). Cutinase Ser 120 is in a crevice between loops 80–90 and 182–189. These loops bear hydrophobic amino acids (Leu 81, Gly 82, Ala 85, Leu 86, Pro 87, Leu 182, Ileu 183; Val 184) which may constitute the interfacial binding site (Fig. 3). The extended active site crevice is partly covered by two thin 'bridges' formed by the side chains of amino acids leu 81 and Val 184, and Leu 182 and Asn 84 C β , respectively (Figs 3 and 4b). Despite these two side-chain bridges and the position of Leu 182, close to HPL Trp 252 and RML Trp 88 (Fig. 2), the catalytic serine of cutinase is not buried under surface loops, but is accessible to solvent (accessible surface of O γ : 5.8 Å², Fig. 4a). The absence of a flap, masking the active-site serine as in other lipases, probably explains why cutinase does not display interfacial activation.

The side chains of the hydrophobic amino acids belonging to the putative interfacial binding site display B-factor values higher than average. This may be an indication that these side chains are mobile and could move upon interface binding. In fact, the simultaneous replacement of Leu 81 and Leu 182 by glycine amino-acid residues, performed on a graphics display, leads to a fully opened active site crevice (compare Fig. 4b and c). This could be the shape of the active-site crevice in contact with the lipidic interface. Brzozowski *et al.*⁴ have proposed that 'the burial of the active site' and 'specific structural changes at the active site' are features necessarily associated with the interfacial activation process. In contrast, the binding of cutinase to interfaces probably does not require a main-chain rearrangement, as in the case of lipases, but only the reorientation of a few lipophilic side chains (for example Leu 81 and Leu 182) that play the role of a 'mini flap'. Nevertheless, the accessibility of the catalytic serine explains the esterase activity of cutinase on short triglycerides (tributyrin, C4) at substrate concentrations below the limit of solubility. Cutinase could therefore interact with the lipid interface more as phospholipases do^{18,19}, even though phospholipases exhibit the interfacial activation process.

The presence of a flap seems closely related to the interfacial activation of lipases^{3–6}. The role of the flap of lipases in substrate docking has been mentioned before⁴; the question now arises about its possible role in the substrate specificity. The activity of lipases and cutinase on triolein, a long C18 triglyceride, follows the order HPL = RML > cutinase, whereas a very similar activity is found towards tributyrin (C4) for the three enzymes (R. Verger, personal communication). This result may be related to the length of the flap and to the depth of the active site (Fig. 4a), which follow the order HPL > RML > cutinase. □

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MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin

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AGONISTS that stimulate protein kinase C (PKC) induce profound changes in cell morphology correlating with the reorganization of submembranous actin^{1,2}, but no direct connection between PKC and actin assembly has been identified³. The myristoylated, alanine-rich C kinase substrate (MARCKS) binds calmodulin^{4,5} and is a predominant, specific substrate of PKC which is phosphorylated during macrophage and neutrophil activation^{6–8}, growth factor-dependent mitogenesis^{9,10} and neurosecretion^{11,12}; it is redistributed from plasma membrane to cytoplasm when phosphorylated^{13–15} and is involved in leukocyte motility^{14,15}. Here we report that MARCKS is a filamentous (F) actin crosslinking protein, with activity that is inhibited by PKC-mediated phosphorylation and by binding to calcium-calmodulin. MARCKS may be a regulated crossbridge between actin and the plasma membrane, and modulation of the actin crosslinking activity of the MARCKS protein by calmodulin and phosphorylation represents a potential convergence of the calcium-calmodulin and PKC signal transduction pathways in the regulation of the actin cytoskeleton.

MARCKS in its phosphorylated and dephosphorylated forms bound to F-actin in a specific and saturable manner (Fig. 1). Both the phosphorylated and dephosphorylated forms of the protein cosedimented with skeletal muscle F-actin at a low stoichiometry of about 1 MARCKS polypeptide per 50 actin monomers (Fig. 1), but the apparent affinity of the dephosphorylated form was more than twice as great as that of phospho-MARCKS (dissociation constants $K_d = 0.28 \pm 0.03 \mu\text{M}$ and $0.86 \pm 0.05 \mu\text{M}$, respectively). MARCKS molecules are rods; the dephospho- and phospho-protein dimensions are $3.7\text{--}5.1 \text{ nm} \times 35.6 \pm 2.8 \text{ nm}$ (mean $\pm$ s.d., $n = 100$) and $2.2\text{--}5.1 \text{ nm} \times$

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29.0 nm $\pm$ 3.0 nm after metal-coating¹⁶, respectively (Fig. 2a and b). These dimensions are in agreement with the high axial ratio of the MARCKS molecule calculated from physical measurements¹⁷ and with the extended helical structure predicted from its amino-acid sequence¹⁸⁻²⁰.

The interaction of MARCKS with F-actin was further studied by electron microscopy after negative staining (Fig. 2c-i). Dephospho-MARCKS but not phospho-MARCKS caused F-actin to aggregate (Fig. 2c-e). At high magnification, filaments in these aggregates are seen to have regions decorated with a cross-hatching of rod-shaped molecules (Fig. 2h and i). Rods occasionally extended to lengths similar to metal-shadowed MARCKS molecules. At a threefold molar excess of dephospho-MARCKS to actin subunits, some regions of the filaments were decorated every ~20 nm and MARCKS molecules could be observed to span short distances between aligned filaments (Fig. 2i). Other regions of the same filaments, however, appeared devoid of MARCKS. This may account for the apparent discrepancy in stoichiometry of binding obtained by direct binding assay (1 MARCKS per 50 actin monomers, Fig. 1) and that of one MARCKS to eight actin subunits calculated to be required to decorate actin fibres with a 20-nm periodicity (arrows, Fig. 2i). The reasons for these 'hot spots' for MARCKS binding on the actin filament are not known: possible explanations include cooperativity of MARCKS binding or specialization of regions of the actin filament. Actin filaments in the absence of MARCKS (Fig. 2c and f) or incubated with phospho-MARCKS (Fig. 2e and g) did not form aggregates and appeared bare along their lengths. Thus, although phosphorylation did not abolish the binding of MARCKS to F-actin (Fig. 1), it did change the way in which phospho-MARCKS bound to F-actin such that it was not visible after negative staining (Fig. 2g).

The effects of MARCKS on actin were further evaluated by

morphological, optical and hydrodynamic methods. Molar ratios of dephospho-MARCKS to actin of up to 1:20 increased the apparent viscosity of F-actin solutions (Fig. 3a); higher ratios, however, reversed this apparent increase in viscosity. These results are consistent with those obtained with other actin-binding proteins²¹ and suggest that low ratios of MARCKS crosslink actin filaments into a network, but higher concentrations aggregate the filaments and prevent the formation of a continuous gel. Studies of the effect of MARCKS on pyrene-labelled actin showed no evidence of an interaction of MARCKS with globular (G) actin nor an effect on the kinetics of filament assembly (data not shown).

Measurements of dynamic light scattering confirmed that dephospho-MARCKS crosslinks F-actin. Dephospho-MARCKS, at a molar ratio of 0.67 to 1 actin, increased the light scattering intensity of F-actin solutions by 110% and decreased the rate of decay of intensity autocorrelation functions (Fig. 3b). Even at saturation, dephospho-MARCKS contributes no more than 2% to the mass of the actin filament, so this increase in light scatter can only be caused by the crosslinking of actin filaments with each other²². By contrast, phospho-MARCKS had minimal effects on the low shear viscosity (Fig. 3a) or light-scattering intensity of F-actin (Fig. 3b). The slight apparent effect of phospho-MARCKS was too small to be reliably measured but was in accordance with its binding mass. These studies (Figs 1-3) demonstrate that dephospho-MARCKS binds to the sides of actin filaments and crosslinks them, but phosphorylated MARCKS binds less tightly to actin filaments and does not crosslink them.

Calcium-calmodulin binds dephospho- but not phospho-MARCKS and prevents PKC-dependent phosphorylation of MARCKS^{4,5,23}. Light-scattering experiments (Fig. 3c) and electron microscopy (data not shown) demonstrate that

FIG. 1 Dephospho- and phospho-MARCKS bind skeletal muscle F-actin. **a**, Actin (2 μ M) was polymerized and incubated for 20 min with the indicated concentration of dephospho- (left) or phospho- (right) MARCKS before sedimentation by centrifugation. The pellet containing F-actin and bound MARCKS was resolved by SDS-PAGE and MARCKS (M) was quantitated by immunoblotting (left) or direct autoradiography of the phosphorylated protein (right). The binding of 120 nM ³²P-labelled MARCKS (—) was competed by a fivefold excess of non-labelled MARCKS (+). **b**, Concentration dependence of binding of dephospho- (open symbols) and phospho- (closed symbols) MARCKS to F-actin; sedimentation in the absence of actin is indicated (diamonds). The non-specific sedimentation, which was never more than 10–12% of the total, was not subtracted from the upper curve. The data represent an average of five independent determinations which did not vary by more than 15%. The data at high MARCKS concentrations (triangles) represent the average of three independent determinations which did not vary by more than 18%. **c**, Scatchard analysis of MARCKS binding to actin; symbols as in **b**.

METHODS. Dephospho-MARCKS was purified from bovine brain and phosphorylated with purified PKC to a stoichiometry of 2.5 mol phosphate per mol MARCKS^{7,17}. Rabbit skeletal muscle actin was purified as described²⁸ with an additional G-150 Sephadex gel filtration step²⁹. F-actin binding was assayed by a co-sedimentation assay³⁰ modified as follows: actin (2 μ M) was polymerized in buffer A containing 100 mM KCl, 2 mM MgCl₂, 0.2 mM CaCl₂, 0.2 mM ATP, 0.2 mM DTT, 2 mM Tris-HCl, pH 7.6. After 1 min at room temperature, the indicated concentrations of MARCKS were added and the incubation was continued for 20 min. Samples (180 μ l) were then centrifuged at 500,000g for 10 min at 20 °C. The pellet, containing roughly 13 μ g F-actin (98% of the total) and bound MARCKS was resuspended and resolved by SDS-PAGE. Phospho-MARCKS was quantitated by Cerenkov counting after excision from the gel, whereas dephospho-MARCKS was transferred electrophoretically to Immobilon and detected by quantitative immunoblotting using a rabbit anti-bovine MARCKS antibody⁶ and ¹²⁵I-labelled protein A. Actin was quantitated by Coomassie blue staining. Similar data were obtained when F-actin was polymerized for 20 min before MARCKS was added.

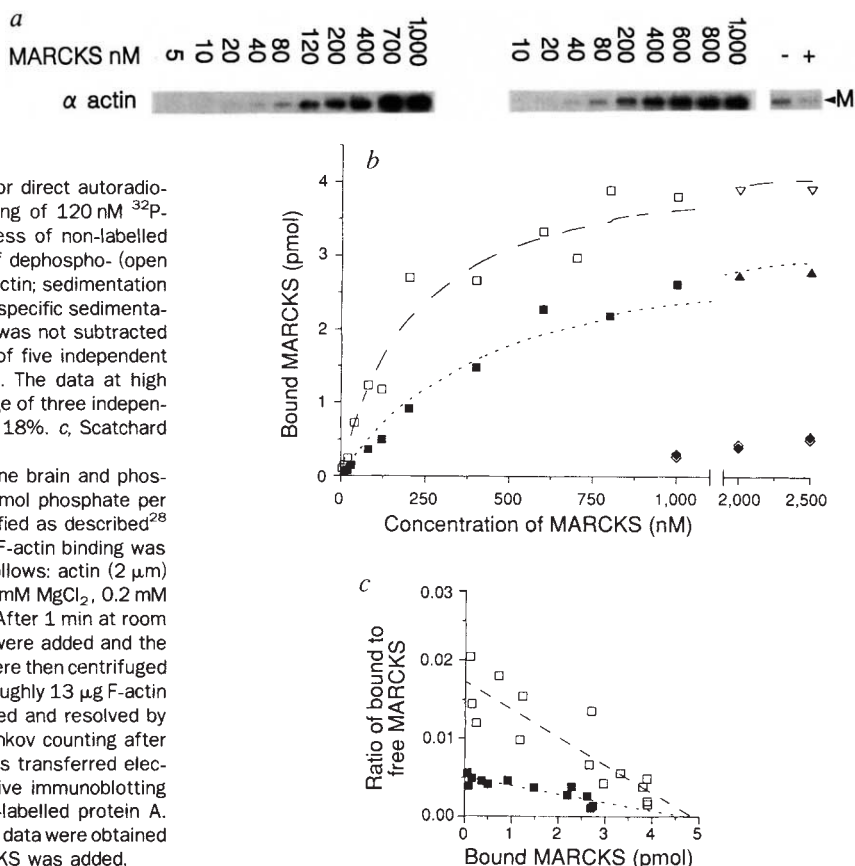
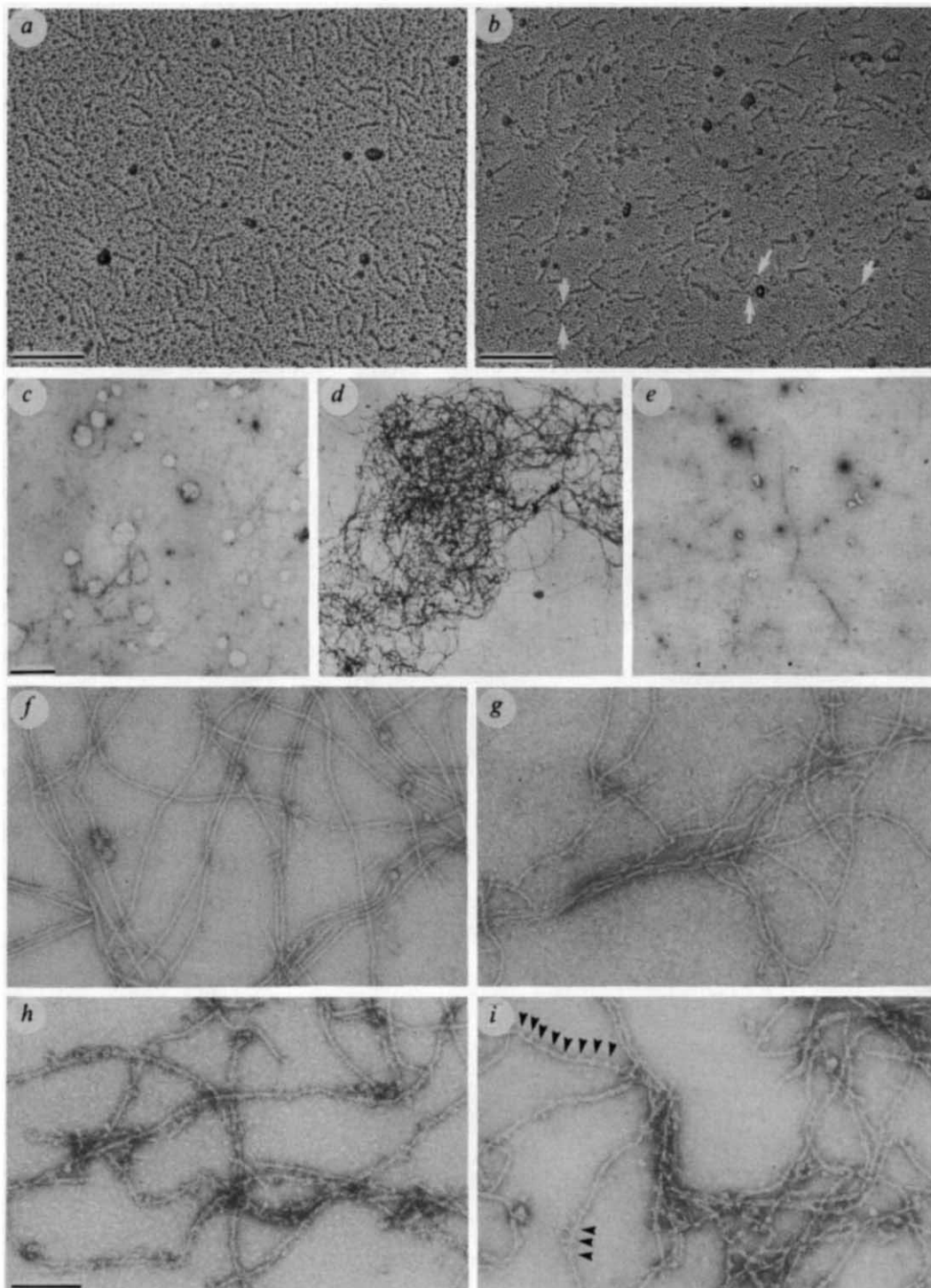


FIG. 2 Structure of MARCKS molecules and effect of MARCKS on the ultrastructure of F-actin. *a, b*, Dephospho- and phospho-bovine MARCKS was adhered to the surface of mica flakes¹⁶, rapidly frozen, freeze-dried, and rotary-coated with 1.2 nm platinum at 5 °C and 3.0 nm carbon at 90 °C. Some phospho-MARCKS molecules appear as two thin strands connected at one of their ends (*b*, arrows). Molecules in both samples occasionally formed end-to-end linear aggregates. Scale bars, 0.1 μ m. *c–i*, The interaction of MARCKS with actin filaments was visualized by negative staining with 2% uranyl acetate. *c–e*, Representative low-magnification micrographs of MARCKS-actin interaction; large aggregates of F-actin are found only in the presence of the dephospho-protein; *c*, 2.4 μ M F-actin (control); *d*, 2.4 μ M F-actin incubated with 0.24 μ M dephospho-MARCKS; *e*, 2.4 μ M F-actin incubated with 0.24 μ M phospho-MARCKS. *c, d, e*, Same magnification; scale bar, 1 μ m. *f–i*, Only dephospho-MARCKS decorates the sides of the actin filaments; *f*, 2.4 μ M F-actin (control); *g*, 2.4 μ M F-actin incubated with 0.24 μ M phospho-MARCKS (higher concentrations of phospho-MARCKS gave similar results); *h*, 2.4 μ M F-actin incubated with 0.24 μ M dephospho-MARCKS; *i*, (2.4 μ M F-actin incubated with 7.2 μ M dephospho-MARCKS. *f–i*, Same magnification; scale bar, 0.1 μ m. At molar ratios of 3 MARCKS: 1 actin subunit, MARCKS can be seen bound along some regions of the fibre surface with a periodicity of 20 nm (*i*, arrowheads), whereas other regions of the same and different filaments appear bare. In marked contrast, phospho-MARCKS molecules are not observed along the sides of the filaments (*g*) despite binding data demonstrating their presence.



stoichiometric amounts of calcium-calmodulin also inhibit the actin crosslinking activity of dephospho-MARCKS, suggesting that at least one actin-binding site, the calmodulin-binding site and the phosphorylation sites of MARCKS are closely linked.

A synthetic peptide based on the calmodulin-binding site of MARCKS also aggregates F-actin. Comparison of bovine¹⁸, chicken¹⁹ and murine MARCKS²⁰ reveals two highly conserved domains: an amino-terminal membrane-binding domain which bears the myristoylation site, and a highly charged phosphorylation domain which also contains the calcium-calmodulin binding site⁴. A synthetic peptide KRFSFKKSFKLSGFSFKKN (single-letter amino-acid code) which corresponds to residues 155–173 of bovine MARCKS¹⁸, containing all the phosphoryla-

tion sites and the calmodulin-binding site^{4,24}, increased the light-scattering intensity of the F-actin solutions (Fig. 4*a*) and aggregated F-actin into loose bundles (Fig. 4*b*). Phosphorylation of the peptide by purified PKC prevented filament bundling, as did the addition of calcium-calmodulin (Fig. 4*a* and *c*).

The sequence of the synthetic peptide used can be modelled as an amphipathic α -helix with the five lysines on one side of the helix and the phosphorylatable serines on the opposite side^{4,5} (Fig. 4, inset). The Lys residues form a consensus calmodulin-binding site²⁵ and this region also resembles the Lys-rich domains of known actin-binding sites²⁶. It is likely that this peptide domain comprises both the calmodulin-binding site and at least one actin-binding site of MARCKS, a hypothesis support-

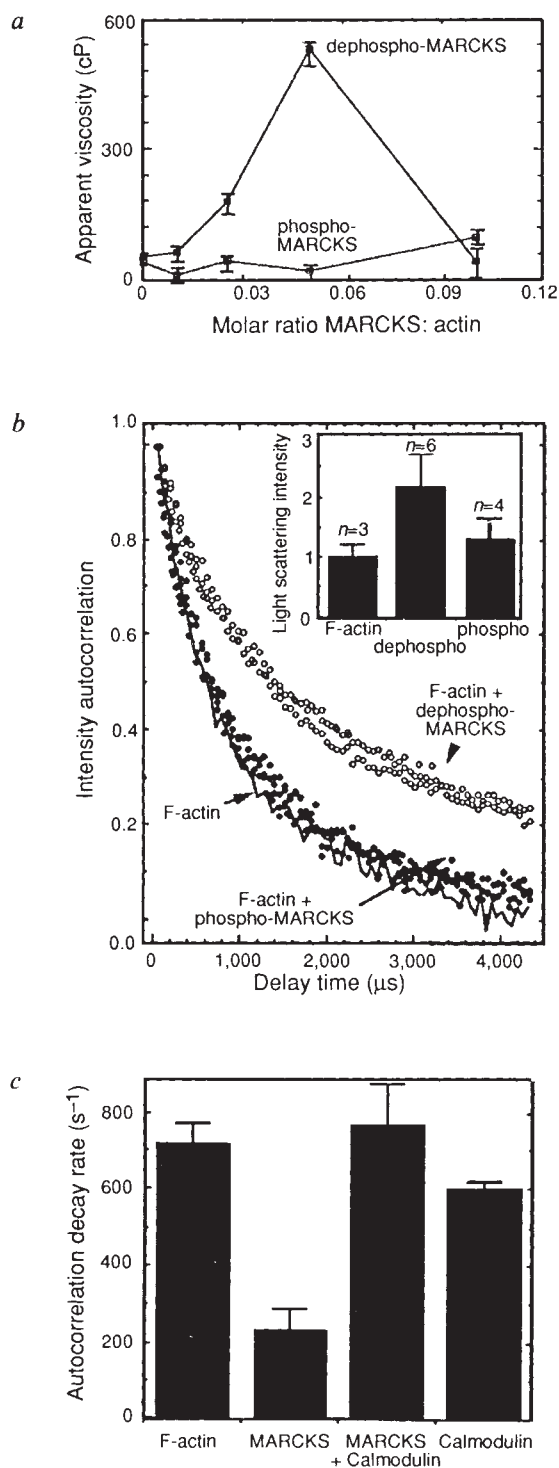


FIG. 3 Interaction of MARCKS with F-actin. *a*, Effect of bovine MARCKS on the low shear rate apparent viscosity of 12 μ M skeletal muscle F-actin in 0.1 M KCl, 0.2 mM CaCl_2 , 2 mM MgCl_2 and 20 mM Tris-HCl, pH 7.5. The bars represent s.e.m. for three measurements. *b*, Effect of MARCKS on F-actin light scattering. The integrated scattering intensity and the intensity autocorrelation of dynamic light scattering³¹ were measured using a Brookhaven BI2030 instrument. Total scattering intensity is normalized to a value of 1 for the control sample containing only F-actin, after subtraction of the scattering from a sample containing only buffer. *c*, Effect of calcium-calmodulin (1.33 μ M) on the light scattered from a 2 μ M F-actin solution in the presence and absence of 1.33 μ M MARCKS. The rate of decay of autocorrelation functions is reported as the initial decay constant derived from a second order cumulative analysis³¹. Only the sample containing dephospho-MARCKS differed significantly from the actin control. The bars show s.d. for three measurements.

ted by the observation that calmodulin inhibits actin bundling by the peptide (Fig. 4*a*). The finding that the peptide can crosslink filaments suggests that it forms dimers or oligomers, and that this process may be inhibited by phosphorylation or by binding calcium-calmodulin.

If the linear polypeptide sequence is related to the MARCKS rod-like structure, attachment to actin filaments would involve a site near the mid-point of the rod. The 20-nm periodicity of filaments incubated with dephospho-MARCKS may arise from this feature. Dephospho-molecules are ~36 nm long; if half of each molecule bound along the side of the filament, the observed

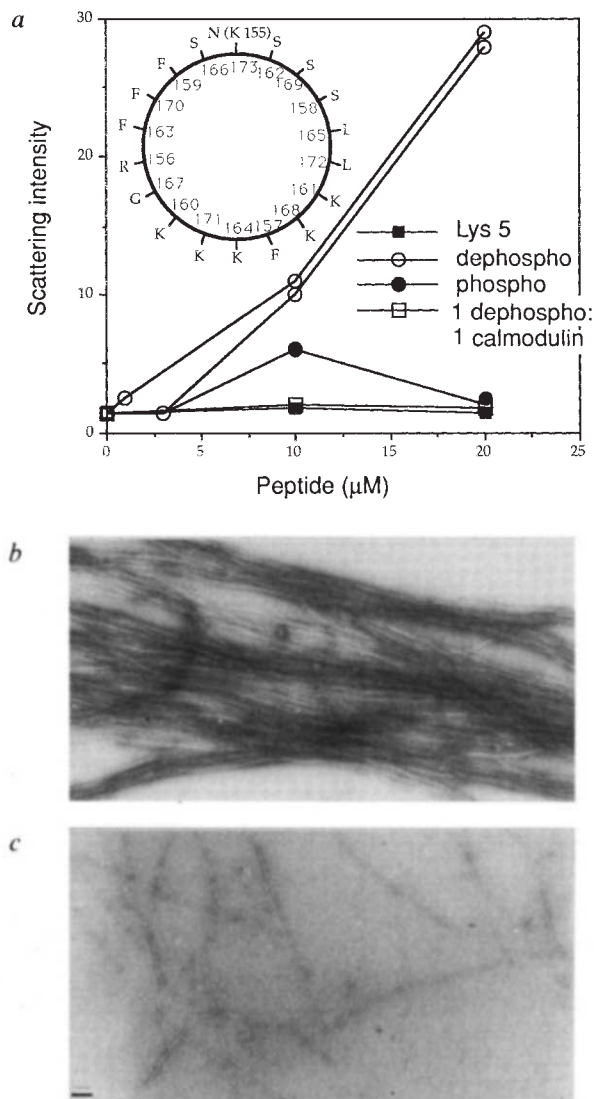


FIG. 4 Effect of a synthetic peptide containing the phosphorylation sites of MARCKS on actin filament structure. The effect of three peptides on actin structure were evaluated: (1) the peptide corresponding to residues 155–173 in bovine MARCKS (KRFSFKKSFKLGSFSSKKN); (2) the phosphorylated version of this peptide, which contains all the serine residues known to be phosphorylated *in vivo*²⁴, was phosphorylated *in vitro* using PKC and contained 2.7 moles phosphate per mole peptide⁷; (3) a pentapeptide of lysine was used as a control because polycationic peptides have been shown to aggregate actin nonspecifically. *a*, Effect of the three synthetic peptides on F-actin light scattering. The inset shows a helical wheel representation of the synthetic peptide. The conditions of incubation were as in Fig. 3. *b*, Structure of actin bundles formed with the dephospho-peptide at a molar ratio of 10 μ M peptide:1 μ M actin. *c*, Phosphorylated peptide (10 μ M peptide:1 μ M actin) does not bundle F-actin. Scale bar, 0.1 μ m.

spacing would occur and protrusion of the rest of the molecule away from the filament would make it visible in the electron microscope. Molecules would, therefore, be seen as L-shapes along the filament. Filament crosslinking would, however, require molecules either to have two actin-binding sites or to dimerize. Phosphorylation would either inactivate one of the actin-binding sites or dissociate the protein to monomers. The loss of mass could account for our inability to detect phospho-MARCKS on actin in the electron microscope although binding itself is only weakly impaired. This model implies that each MARCKS polypeptide has only one actin-binding site and that phosphorylation or association with calcium-calmodulin disrupts MARCKS-MARCKS interaction. Alternatively, MARCKS might have two actin-binding sites, only one of which is regulated by phosphorylation and/or by calcium-calmodulin.

Cytoskeletal rearrangement during neurosecretion, leukocyte activation, and growth factor-dependent mitogenesis is accompanied by the rapid, PKC-dependent, phosphorylation of MARCKS⁶⁻¹². Phosphorylation displaces MARCKS from the plasma membrane and subsequent dephosphorylation is accompanied by reassociation with the membrane^{6,13-15}. In macrophages, MARCKS is found in focal contacts where actin filaments attach to the substrate-adherent plasma membrane, in association with vinculin, talin and PKC (ref. 14, and A.R., J.H., M.T., A.C.N. and A.A., manuscript submitted). Chemotactic stimulation of leukocytes, resulting in both the activation of PKC and increased levels of cytosolic free calcium^{8,27}, generates two signals that can modify the interaction of MARCKS with actin and the attachment of MARCKS to the plasma membrane. MARCKS might therefore provide a dynamic, PKC- and calcium-calmodulin-regulated crossbridge between the actin cytoskeleton and the substrate-adherent plasma membrane during chemotaxis. □

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Sex and sensibility

David L. Hull

The Ant and the Peacock: Altruism and Sexual Selection from Darwin to Today. By Helena Cronin. Cambridge University Press: 1992. Pp. 490. £27.50, \$39.50.

In his foreword to *The Ant and the Peacock*, John Maynard Smith applauds Helena Cronin for resisting the "recent fashion in the history of science . . . to ignore the science, but to describe in sordid detail the political tactics of the scientists". But Cronin's book itself is one more fusillade in the conflict between what Cronin calls modern darwinism and its pluralist opponents. Modern darwinians like Richard Dawkins, Mark Ridley and Maynard Smith emphasize the central role in evolution of genes, selection, adaptation and evolutionary stable strategies, whereas such pluralistic opponents as Richard Lewontin and Stephen Jay Gould argue that selection occurs at a variety of levels and ridicule what they take to be the panglossian adaptationism, panselectionism and genetic determinism of the so-called modern darwinians. Cronin compares her own reading of Dawkins' *The Selfish Gene* to crossing Wallace's line, the narrow but deep channel running between Bali and Lombok that marks the sharp separation between the fauna of Asia and Australia. "Here was a different world. Darwinism had entered a new era."

Cronin's book is a mixture of careful, perceptive history and enthusiastic advocacy, just the sort of history that present-day historians denounce as 'Whiggism', a term that is rivalled only by 'positivism' in the current lexicon of intellectual bad habits. According to historians, the relationship between knowledge of past and present science is not symmetrical. Present-day scientists can benefit from reading the history of their field, but the introduction of current knowledge into discussions of past episodes in the history of science is sure to distort our understanding. If Whiggism is the evil that so many historians claim it is, Cronin's book should be a disaster. It is anything but. On the contrary, she succeeds in keeping the past and present separate when she needs to and in illuminating past controversies by introducing current thoughts on the subject. In her exposition, the light of understanding shines in both directions.

Cronin investigates two issues in evolutionary biology — altruism as represented by the selfless sacrifice of neuter insects such as soldier ants, and sexual selection as represented by the male peacock's tail. She not only traces the

history of these two sets of interrelated problems from Charles Darwin and Alfred Russel Wallace to the present, but also argues for the virtues of certain solutions over others. It is difficult to think of two more fascinating topics than altruism and sexual selection. Goodness and sex are an unbeatable combination. These topics also serve to make clear the

that they did not possess. Although Wallace initially allowed that female choice might have some effect, he thought natural selection acting differently on males and females was the primary cause of sexual dimorphism. Eventually both Darwin and Wallace were forced to postulate nonadaptive mechanisms as well; hereditary tendencies for Darwin, physiological processes for Wallace.

The issue of female choice languished for over a century until proponents of modern darwinism resurrected it. As Cronin points out, when viewed from the gene-centred perspective of modern darwinism, the dispute between Darwin and Wallace over sexual selection becomes a tempest in a victorian teacup.



Not just a pretty tail: detail from the painting *Peacock and Peahen* by Tobias Stranover (1684–1731).

differences that divided Darwin and Wallace, and for a change Wallace gets his just due. Where can one find the most sophisticated and penetrating discussions of the evolutionary process prior to the rebirth of darwinism in this century? In the exchanges between Darwin and Wallace.

Darwin viewed sexual dimorphism as posing special problems for natural selection. How could extravagant secondary sexual characteristics such as the male peacock's tail be explained in terms of natural selection? Darwin suggested two mechanisms for such characteristics: the competition between members of the same sex (usually males) for mates, and mate choice (usually female choice). Darwin's contemporaries saw little problem with male combat. The formidable antlers possessed by males in the deer family, for example, are clearly used in battles for access to females. But female choice was quite another matter. It seemed to attribute abilities to organisms

The problems that Darwin and Wallace saw arise only when one concentrates on organisms instead of genes. From the gene-centred perspective of modern darwinism, sexual selection is merely a special form of natural selection.

"For Aesop, the social insects were a source of inspiration. For Darwinians, they were a source of aggravation", writes Cronin, but for the darwinians the aggravation did not stem, as it does today, from the altruistic behaviour of neuter insects, but from their remarkable adaptations. How could soldier ants develop such huge mandibles when they never reproduce themselves? Darwin insisted that no adaptation in one species can serve primarily to benefit organisms belonging to some other species, but he did not see intraspecific altruism as a serious problem. According to modern darwinians, it is. Any genes that lead an organism to behave in ways that decrease the likelihood that these genes will be passed on to later generations

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should be rapidly eliminated.

According to Cronin, neither Darwin nor Wallace saw intraspecific altruism as a problem because they slid half-consciously from the good of organisms to the good of communities, colonies and even species, an elision that G. C. Williams finally brought forcefully to our attention. If treating organisms as units of selection is mistaken, then treating higher-level entities as units of selection is sure to lead to problems. Cronin agrees with Dawkins that genes can function as "replicators whereas organisms, groups and other levels in the hierarchy cannot. Natural selection is about the differential survival of replicators. So genes are the only serious candidates for units of selection." Here I think that Cronin reasons too quickly. Replication is necessary for selection but is not equivalent to it. Organisms are more than 'vehicles'.

Cronin also addresses two other differences between the views of Darwin and Wallace — the role of selection in speciation and the adequacy of a purely naturalistic theory to explain the moral and intellectual attainments of the human species. Wallace was fond of proclaiming that he was more of a darwinian than was Darwin. In the case of speciation he was right. Wallace thought that intersterility arose as an adaptation through natural selection, whereas Darwin thought that it was merely an incidental effect of selection acting on other characteristics. But with respect to the human species, Wallace was anything but a darwinian, because he appealed to supernatural causes. In fact, Darwin and Wallace disagreed with each other so profoundly on so many issues that it seems more than peculiar to claim that these two men were authors of the same theory. They agreed that species evolve but disagreed on just about every other particular of the theory, including the scope and efficacy of natural selection. Cronin points out these differences more dramatically than any previous writer, partly because she has not been afraid of using our current understanding to explain the past. □

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■ In June 1858, Darwin received Wallace's now famous letter, enclosing an essay in which Wallace described his own theory of evolution. "I never saw a more striking coincidence", Darwin wrote to Charles Lyell. "So all my originality, whatever it may amount to, will be smashed." The letters surrounding these events and the eventual publication of the abstract of Darwin's theory a year later appear in *The Correspondence of Charles Darwin: Vol. 7 1858-1859*, recently published by Cambridge University Press. Price £35, \$59.95.

Nervous starts

J. Z. Young

Foundations of the Neuron Doctrine. By Gordon M. Shepherd. *Oxford University Press: 1992. Pp. 338. £35, \$39.95.*

Cajal's Degeneration and Regeneration of the Nervous System. Translated by R. M. May. Edited by Javier De Felipe and Edward G. Jones. *Oxford University Press: 1991. Pp. 769. £60, \$65.*

THOSE who probe the nervous system with electrodes probably seldom stop to consider the history of knowledge of the cells they are impaling. Yet it would help them to think about the problems that have arisen in the search for units of nervous activity. Since the days of Santiago Ramón y Cajal, most neuroscientists have depended on a rather simple



Cajal: proponent of "connection by contact".

picture of the neuron, with dendrites, cell body and axon as the essential unit. This has also been the model mostly used in artificial intelligence. Shepherd's book provides a survey of the history of the neuronal hypothesis. In his last chapter, he raises the question of whether we should now look for units both larger and smaller than the neuron.

The controversy at the end of the last century turned on the question of whether neurofibrils proceed from one cell to the next. It was conducted in fairly ferocious language. Cajal writes of his reticularist opponents, such as Golgi and A. Bethe, as "fanatics with haughty minds, inclined towards mysticism". Finally, in 1917, he is happy to write that "the unhorsed physiologist of Strasbourg [Bethe] decided to abandon the field. *Victis honos!*"

Cajal was, of course, correct in claiming that "connection is by contact", but his opponents were skilful light microscop-

pists and not so far wrong as he supposed. Now that electron microscopy has shown the correct relationships at synapses, we can see that their interpretations were in a sense correct. The finest branches of a nerve fibre may indeed appear to enter the end organ, for instance in the groove at the surface of a muscle fibre. There is no evidence that Cajal realized that it is the completeness of the two membranes that is important. If the finer branches run in a trough, the most honest light-microscope interpretation may be that there is continuity. Cajal's opinion was right, but his figures are almost all drawings.

The advocates of the neuron theory were themselves quite "haughty" and hasty in their rejection of all possibilities of "continuity". We know now that gap junctions may allow passage of ions and small molecules between neurons. Furthermore, there may be complete fusion of nerve cells if they always function together. For instance, the two giant cells of the squid initiate contraction of the muscle sac — and they are completely joined by a bridge: for jetting, both sides of the mantle must contract together. But where impulses are initiated there are synapses. This is a system of "Fused neurons and synaptic contacts", as the paper in which it was described was called in 1939. The fusion is the exception that proves the rule. Nerve fibres *can* fuse, but where decisions are to be made they are separated by synapses. I remember explaining all this to Sherrington (in about 1938). He looked up at me quizzically and said, "I hope that you are right Young, but I find it hard to believe." It is ironic that the squid's giant fibre synapse, more thoroughly investigated than any other, involves a syncytial postsynaptic fibre. I hope that Cajal would have enjoyed the joke (but I'm not sure that he would).

This history of old doubts and quarrels shows how hard it is to arrive at secure knowledge. As more has been discovered it becomes clear that the classical neuron doctrine needs to be extended. Almost from the start there were doubts as to what the term should include. The word 'neuron', originally suggested by Waldeyer in 1891, comes from the Greek, meaning, literally, tendon or sinew, and was applied through confusion to nerve trunks. Some authors therefore wished to keep the term neuron for the axon, whereas others (paradoxically) tried to use it only for the nerve cell body. Kölliker and others emphasized that the word should be spelled 'neurone'. This usage is still insisted on by some British physiologists and by Cambridge University Press. Shepherd nowhere mentions the history of this spelling. Many people must be puzzled to know which form to use and

the book could have given authoritative guidance. Surely British physiologists and Cambridge University Press should now abandon this pretentious and unhelpful practice and follow the rest of the world.

More serious are the problems raised by the discovery that dendrites may have synaptic outputs and that axons can have inputs from other axons. Moreover, dendrites do not always have graded synaptic responses but may carry voltage-gated propagated action potentials, whereas, conversely, some axons do not carry these at all. Shepherd summarizes the effects of such processes: "there is not a fixed correlation of structure and function within the different parts of the neuron; axons and dendrites provide flexible substrates in which a variety of membrane channels and local organelles . . . can support different types of physiological properties and function operations . . . So although the neuron remains a basic anatomical, physiological, genetic and metabolic unit . . . it contains several levels of local subunits, and is itself a part of larger multineuronal units." Such a neuron has several potentially modifiable parts. It will provide a truer picture for neuroscientists and theoreticians who are trying to model parallel computing systems (although there will be difficulty in constructing them).

It is useful to have, at the same time as this review of the neuron doctrine, a new issue of Cajal's own book on degeneration and regeneration of the nervous system. This was first published in Spanish in 1913–14, the cost of publication being covered by expatriate Spanish physicians in Argentina in honour of Cajal's Nobel prize. It was translated into English in 1928, but without several important sections that are included in the present edition. Complete with Cajal's excellent pictures of his preparations, the new edition makes a wonderfully full account of regeneration. Several of his most important ideas are developed here. Particularly relevant to modern work are the concepts of neurotrophism and his studies of regeneration in the central nervous system. It is good to have this book, but it tempts one to complain that Cajal's greatest work, *Histology of the Nervous System of Man and the Vertebrates*, published in 1899 in Spanish and in 1909 in French, is still not available in English. Illustrated again by Cajal's beautiful figures, that book provides detail of every part of the brain and peripheral nervous system, and should be accessible to every neuroscientist. □

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The world's genetic libraries

Paul Colinvaux

Diversity and the Tropical Rainforest. By John Terborgh. *Scientific American Library/W. H. Freeman*: 1992. Pp. 242. \$32.95, £17.95.

BIOLOGY textbooks talk of rainforests as 'layered', five layers being the favoured number. This postulate (it has been little more) dates to Paul Richards' book *The Tropical Rainforest* (Cambridge University Press, 1952), the first real compendium of rainforest phenomena. But can plants staked out under the Sun really be expected to form layers, each plant, as it were, assigned to its proper station?

In a fine overview of rainforest law, John Terborgh offers an answer based on the geometry of light and shadow. Tree tops are crude cylinders, inevitably with gaps in between. Light angles down between them, eventually to blend and provide modest but even illumination throughout the day. Shaded trees get their best net energy returns by spreading their canopies in this even light. Add a layer for the darkness of the forest floor, and another for emergent trees as a strategy made possible by year-round productivity, and four-fifths of Richards' layers are explained.

But the greatest oddity of rainforests is their astonishing diversity. The latest spectacular assessment comes from the gassing by Terry Erwin of the canopies of trees of a single species in Panama. This brought down 1,200 species of beetle, most of them new to science. If 13.5 per cent of these beetles were host-specific, if as many of all arthropods were equally host-specific, and if all 50,000 known species of tropical forest tree had an equivalent insect ration, then the world's rainforests hold 30 million species. If, if, if . . . Nobody actually believes this extrapolation. Perhaps the real figure is a mere three million.

For the trees themselves, Alwyn Gentry made the greatest tally yet, on the lower Rio Napo in Peru. Gentry climbed every tree in a hectare (10,000 square metres) to sample flowers, fruits and leaves. The perilous climbing, with ropes and irons, 20–50 metres into the green mists overhead, took him more than a month. After a year or more of waiting for all of the specialist taxonomists to make their reports, Gentry had shown that 300 species were included in the 606 trees with trunks 10 centimetres or more in diameter. Terborgh notes that Gentry was without assistants because no-one would fund



Light and shade encourage development of a layered forest. (Picture is taken from *The Last Rain Forests* edited by M. Collins and published by Mitchell Beazley. Price £17.99.)

this vital work at a sensible level.

How does life in the wet and warm, with no winter, and the Sun at its zenith, allow the packing together of so vast an array? Whole new ways of life are possible, guilds of ant-following birds, for instance, and guilds of fruit and nectar feeders. The area of land for specialism in any temperature regime is far larger at the equator than at high latitudes, theoretically allowing fine separations between species. And so on.

But the most persistent attempts to explain the high diversity of life in rainforests have used the idea of differential extinction. High latitudes have lost their species to extinction in ice ages or from harsh seasonal vicissitudes. Low lati-

tudes have not only been spared but have avoided competitive extinction because of the 'intermediate disturbance' of falling trees, or the dispersal forced by predators. Both processes should prevent winner-take-all competition from driving the losers from existence.

Only one current hypothesis invokes the more fundamental idea of enhanced speciation in the tropics, the so-called refugial hypothesis of Jurgen Haffer. This hypothesis seeks to explain disjunct distributions of Amazon birds and butterflies by imagining a time of aridity in the last ice age that should have divided the Amazon forest, letting speciation proceed in forest fragments as it does on islands in the sea. On this thinking, a speciation episode should happen with every glacial cycle, as the forest was first broken, then remerged, with the changing of the rains.

Terborgh lists only two "faint" voices that doubt the reality of this model, John Endler's and mine. Endler points out the gross lack of parsimony, whereas I base my whimpering on testing the proposed history with palaeoecological data. Initial results from the ice-age Amazon suggest cooling, with concentration of the forest in the supposedly arid lowlands.

Terborgh develops a new insight from the discussion, at once novel and appealing. Vicariance events (that is separation into 'other' isolated populations before

remerging) need to be of the order of 100,000 years long for critical genetic changes to take place. Ice-age cycles are of this order. The pacemaker of ice ages is perturbation of the Earth's orbit and tilt, an effect known to palaeoclimatologists as Milankovitch climatic change. Terborgh suggests the tantalizing possibility that most speciation since the dawn of life has been driven by Milankovitch-induced vicariance cycles. Fine though the idea is, a sceptic would contend that Milankovitch climatic change, outside glacial cycles, is too subtle for the requirements of vicariance.

This book is the most accessible, and best, review of the properties and intrigue of rainforests. Tales are told about Terborgh and his prowess as an Amazonian biologist. He writes with the passion of unexcelled knowledge about the wonders and possibilities of rainforests. His prognosis for their future in a world beset by excess human reproduction is sober — essentially, he believes that the forests cannot survive without cultural changes in human societies, changes that will take too long. In the name of whatever gods may be, give us money and people to catalogue and question in the last decades before the forest is all gone. □

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The flight of the speedy bee

Thomas D. Seeley

Killer Bees: The Africanized Honey Bee in the Americas. By Mark L. Winston. Harvard University Press: 1992. Pp. 155. \$19.95, £15.50.

SOME 35 years ago, several dozen queen honey bees from Africa were imported into Brazil for cross-breeding with other honey bees already there, with the aim of producing a bee well suited to tropical conditions. Shortly after its introduction, some of the African stock escaped and became established as a feral population in central Brazil. From here, the population spread roughly 500 kilometres a year, colonizing most of tropical and subtropical America and displacing other subspecies of the honey bee, previously imported from Europe. At present, the colonizing front of the Africanized bee is within the state of Texas, and over the next several years it is expected to spread throughout the southern third of the United States, stopping only when it reaches regions with harsh winters.

Long before the Africanized bee

reached North America, it had been nicknamed 'the killer bee', with journalistic reports, books and films produced in the United States all focusing on lurid tales of death by stinging, and causing considerable alarm. Now that the bee is in the United States and there to stay, it is time for more responsible journalism. In *Killer Bees*, Mark Winston encourages the spread of sound information about the Africanized bee by providing a concise, factual introduction to its biology. This book is not intended to serve as a detailed scholarly review for specialists (for this, one should consult two recent books: *Africanized Honey Bees and Bee Mites*, Ellis Horwood, 1988 and *The "African" Honey Bee*, Westview Press, 1991, the former by Glen Needham *et al.*, the latter by Marla Spivak *et al.*); rather, it is aimed at general readers such as local government officials and the public, and the commercial beekeepers who will deal daily with the bee.

The book consists of two parts. In part one, "Biology and Habits", Winston draws on his years of first-hand experience with Africanized bees in French Guiana, Venezuela and Peru to describe these insects' traits and to explain their adaptiveness for tropical life. The reader learns, for example, that the feistiness of this bee has its roots in millions of years

of intense predation in Africa by such adversaries as ants, honey badgers and especially humans. Hence, ultimately, the problem of the Africanized bee is one of our own making. More importantly, Winston explains that the principal problem that beekeepers will face is the bee's tendency to swarm (that is, to undergo colony fission for reproduction), to build small colonies and to store only a little honey. Beekeeping in North America currently relies on bees of European origin, which were shaped by natural selection to swarm infrequently and to form large colonies with large honey stores, thereby enabling them to survive cold winters. By contrast, the traits of Africanized bees are superior where there is no hurdle of winter survival, but where there is a premium on rapid reproduction to exploit new or under-used habitats.

In part two, "Impact and Control", Winston reviews the consequences of the Africanized bees' traits for the general public, but especially for beekeepers. Here he draws upon his experience with the beekeeping industry of Canada. This part is likely to be controversial, for Winston foresees the Africanized bee having "a devastating effect on our beekeeping systems". He points out that some 40 per cent of the 3 million honey-bee colonies in the United States are moved each spring from the southern to the northern states, in accordance with the timing of different crops that need pollination or offer honey production, and that such large-scale bee movement would spread the Africanized bee beyond its natural range within the southern states. To reduce the impact of Africanized bees in the United States. He recommends prohibiting migratory beekeeping between areas with and without the Africanized bee. Also, given the difficulty of managing colonies of the feisty and swarm-prone Africanized bee, Winston recommends mandatory annual requeening of all beekeepers' colonies in Africanized zones, using certified queens of European descent. This would require shifting the production of queen bees from its current setting, in the southern states and California, to parts of the world without Africanized bees — Europe, the northern United States, and islands such as Hawaii.

People working within some parts of the US beekeeping industry may be irritated by recommendations like these. Nevertheless, as a unified account of the biology and impact of the Africanized bee, the book must be reckoned with by anyone involved with the immigration of this insect into the United States. □

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Death of a salesman?

David M. Ritson

Teller's War: The Top-Secret Story Behind the Star Wars Deception. By William L. Broad. Simon and Schuster: 1992. Pp. 380. \$25.

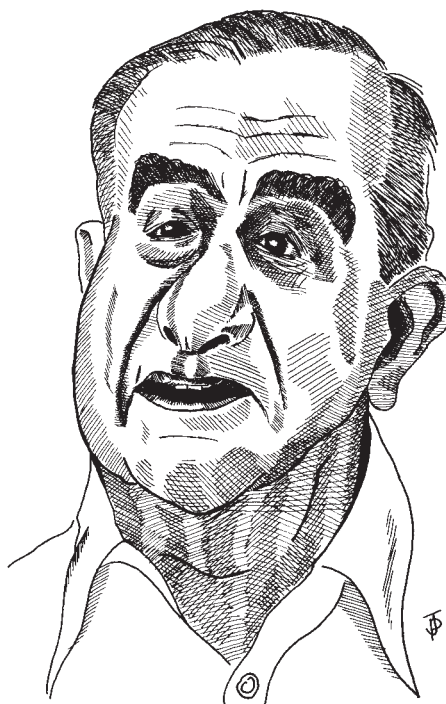
In 1985 William Broad, science correspondent for the *New York Times*, wrote *The Star Warriors*, describing his seven-day visit to the Lawrence Livermore National Laboratory to research a story on the fission bomb-driven X-ray laser project. He was a guest at the home of Lowell Wood, a division head, and was sold on the project mainly by Wood's vision and flair for public relations. Now, in what in many senses is a follow-on book, Broad presents an obsessively researched and documented chronicle of the pathological oversell of this component of 'Star Wars'. Who better than a reformed alcoholic to preach the dangers of alcohol?

It is hard now to recall how controversial the Strategic Defense Initiative (SDI) was only a few years ago and how, under the cover of almost complete government classification, it was so difficult to distinguish fact from fiction in the scientific claims made for the various weapons systems. Centre-stage in the controversy for several years were Edward Teller and the X-ray laser, followed by "Brilliant Pebbles", in which thousands of small independent interceptors would be stationed in space. Teller's reply to criticism at the time was invariably "if you only knew what I know". Now, with Broad's research, we can indeed all know both what was known and what was claimed. And not surprisingly, the claims went outrageously beyond the facts.

What made all of these revelations possible were the grievance hearings brought before the University of California at Berkeley in 1987 by Roy Woodruff. As an associate director for nuclear weapons at Livermore, Woodruff had been directly responsible for supervising the X-ray laser programme. He resigned from the post in October 1985, alleging that the project had been grossly misrepresented. During the hearings, he complained of reprisals and penalization for his stance. The hearings were leaked, providing a great deal of information about the project. The demise of SDI in its 1980s manifestation has also made many of the main protagonists ready and willing to discuss the project both on and off the record. Broad has exploited the situation essentially to declassify the X-ray laser component. The resulting book is fascinating and addictive.

Its weakness lies in its simplistic depictions and plot. Broad characterizes Teller as the principal architect of SDI and

portrays the X-ray laser as though it were the main component of the programme. In fact, the X-ray laser programme cost about 2 billion dollars out of a total expenditure on SDI in the 1980s of around 25 billion dollars. There are three main players in the book. Teller: "Largely divorced from dispassionate peers, Teller drew on the vast reservoir of his own ideas, dreams, passions and frustrations. Foremost among his frustra-



tions was the military might of the Soviet Union." Wood: "a youthful colleague who provided no moderation at all but seemed to goad Teller onwards . . . Wood was an extension of Teller, a protégé, an intellectual devotee who pledged allegiance to the Tellerian creed, and struggled to carry it forward." And, pitted against the two, is Woodruff: "Young and idealistic, headstrong and unfamiliar with the corridors of scientific moderation of which he was only partly aware. He was ready to bring the visionary Teller down to earth." A subplot running through the book is the representation of Teller: "Most fundamentally the trouble in Teller's life can be traced to a breakdown in relations with colleagues . . . Teller needed peers to give integrity to the wanderings of his extraordinarily vivid imagination. Bereft of interaction with colleagues, he was an undisciplined dreamer." It is, of course, true that even

after all the other protagonists in the 1954 US Atomic Energy Commission hearings concerning Oppenheimer's opposition to the development of the H-bomb are forgiven or forgotten, the physicists of that era have never forgiven Teller. But that generation has now all but exited from the stage, and the principal actors today hardly remember those times. Probably a far more important problem is the immense ego that has always driven Teller, which must make it hard for him to brook contradiction.

Broad does an excellent job of portraying the travails of a 'whistle blower'. Woodruff was plagued before finally leaving Livermore by multiple investigations, a cut in salary and isolation from his colleagues. What is missing, however, is an account of the wider arena of the Star Wars programme, and of the forces that drove it and, for that matter, continue to do so as it changes (at an ever increasing cost) to become a shield against attack from Saddam Hussein and his ilk. It is comforting to believe that Star Wars was simply an aberration in Teller and Reagan's judgements, with one autocratic brilliant old man selling another less-brilliant old man on a joint dream. This is indeed the picture that Broad paints. The reality, when it finally comes to be written, is likely to be more interesting and more disquieting. Many members of the Reagan administration were tough, intelligent and pragmatic individuals, motivated by their discontent with military parity and a desire to re-establish US superiority in that respect. Under the circumstances, it is hard to determine to what extent Teller used the system and to what extent it used him.

In his epilogue, Broad raises but fails to answer many of the more interesting questions, such as how a responsible director at Livermore could have tolerated such flagrant misrepresentations by its public-relations people; why the system of checks and balances did not function better; and where the University of California's management and supervision were through all of this. My own cynical view is that the scientific community can deal very well with scams such as cold fusion where only millions of dollars are at risk, but that it collapses over big projects where billions or hundreds of billions of dollars are in question. For its wealth of new facts and careful documentation of the science behind SDI, Broad's book deserves to be widely read. □

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Parnassus in the living room

John W. Galloway

Houses for Science: A Pictorial History of Cold Spring Harbor Laboratory. By Elizabeth L. Watson. Cold Spring Harbor Laboratory Press: 1992. Pp. 352. \$75.

THIS must be a first. It is certainly the first coffee-table book about a scientific institution that has come my way, and a good thing too. Coffee-tables apart, *Houses for Science* is rather difficult to pigeonhole, which is no bad thing either. It looks like a labour of love by Elizabeth Watson, who works on the preservation of historic buildings, including those at Cold Spring Harbor Laboratory. James D. Watson happens to be her husband, and for nearly a quarter of a century he has been the director there.

The laboratory's contributions to genetics and molecular biology are not disputed. Its corporate intellect has been, and I dare say still is, formidable — Delbrück, Hershey, Luria and McClintock all worked there. But the main charm of the place is the combination of its beautiful situation on Long Island's north shore and its buildings. As with other institutions — and like Topsy in *Uncle Tom's Cabin* — it seems to have 'just grown'; but whereas the architecture of many institutions resembles nothing so much as a termites' nest, at Cold Spring Harbor Laboratory there has been plenty of space and money for building, at least for some of the time. This has led to an agreeable mixture of nineteenth- and early twentieth-century vernacular architecture and fairly self-contained purpose-built laboratories, lecture theatres and so on.

Some of the buildings were once the houses of the affluent who used to live there. Others are greatly restored derelict buildings that were the remnants of the site's industrial past. Cold Spring Harbor was a whaling station until the discovery of petroleum saved the whale — and sank the whalers. Before the Yankee whalers arrived, Indians of the Matinecock tribe had encamped at the head of the harbour; the infinitely attractive quartz heads of their arrows and spears still litter the site. Matinecock homes, being rather more biodegradable than those that came later, do not now house laboratories, although these early settlers' long but all too easily loosened tenure of the land is nodded to in the Wawepex building, which takes its name from an Algonquian word meaning "at the good little water-place".

The book is not only a history of the laboratory seen through its buildings,

but also an architectural primer, containing a short pictorial glossary illustrated with examples from Cold Spring Harbor Laboratory. We are also taught architectural history, for instance about the Palladian roots of the Georgian style. And we learn that Sammis Hall of residence was closely modelled on Palladio's Villa Poania at Vincenza. To find information such as this is a pleasant surprise, and occasionally thought-provoking. After all, a considerable period separated sixteenth-century Palladian architecture from the eighteenth-century Georgian era; yet John Summerson's 'Georgian London' confirms the influence of the one on the other.

The history of the laboratory's build-

ford invited him to visit the Cold Spring Harbor fish hatchery. A local businessman and landowner, John Divine Jones, offered them buildings on the sea front (dare we say that Divine intervention played a part?), and in 1890 the biological laboratory was launched. Later, the Carnegie Institution built a genetics laboratory on the site, Woods Hole having resisted their blandishments. And much later the two merged to become the laboratory as it is today.

The story told in the book is helped by the inclusion of a potted history of the main movements in modern molecular biology and genetics, presented in the form of short essays by James Watson. The writing of such essays is a difficult



Panoramic view of Cold Spring Harbor painted by Edward Lange in the 1880s. Bungtown Barrel Factory can be seen on the western shore (left) and the grist mill is visible on the eastern shore (right).

ings is interspersed with the history of its science, as well as some of its politics. It is always reassuring to discover that the great in science are no different from the rest of us, having the same clay feet but just happening to be better scientists. Take, for example, the Nobel prize-winner Edward Tatum, for a while the laboratory's chairman, who brought it to the brink of financial disaster in the early 1960s until John Cairns became director.

As with much of history, the birth of Cold Spring Harbor Laboratory can be put down to chance, overfishing being the ultimate reason that the laboratory is around today. In response to overfishing came conservation and its practical arm, fish farming. Eugene Blackford, who sat on the board of the New York Fisheries Commission, also sat on the board of the Brooklyn Institute of Arts and Sciences. Fellow academy member and zoology professor Franklin Hooper was keen to set up a second US marine zoological station modelled on the numerous ones established in Europe — France already had six — and on the one already at Woods Hole in Massachusetts. Black-

ford invited him to visit the Cold Spring Harbor fish hatchery. A local businessman and landowner, John Divine Jones, offered them buildings on the sea front (dare we say that Divine intervention played a part?), and in 1890 the biological laboratory was launched. Later, the Carnegie Institution built a genetics laboratory on the site, Woods Hole having resisted their blandishments. And much later the two merged to become the laboratory as it is today.

It is a pity to have to complain, because the book is both handsome and informative. The placing of scientists and their families into small suburban domestic and commercial buildings gives science the feel of a real enterprise carried out by real people. This is far removed from the abstract, depersonalized activity that science so often comes across as and, shamefully, at which scientists themselves traditionally connive. □

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A Valhalla of science

Ed Regis

Millikan's School: A History of the California Institute of Technology. By Judith R. Goodstein. Norton: 1991. Pp. 317. \$25, £17.95.

As befits a book about an institute of technology located in southern California, *Millikan's School* first comes to life — one might say erupts — in a chapter about earthquakes. The initial chapters brim over with dull administrative detail, paragraph after paragraph of who contributed how much money to the growing school, of how many acres of orange groves were sacrificed to classroom buildings, and so on, to the comparative neglect of the grand purpose of it all, which was the further advancement of science. But suddenly, a hundred pages into the story (and not a moment too soon), earthquakes burst upon the scene with all the impact of a magnitude 6 event on the Richter scale.

Charles Richter was, in fact, one of the leading lights of Caltech. He first visited the campus in the early 1920s and heard Robert A. Millikan lecture on physics. So impressed was he with Millikan's talk that Richter, who had an undergraduate degree from Stanford, left his job as a warehouseman and enrolled as a graduate student in physics. Not long afterwards, he was seeing Albert Einstein bicycling around the campus, listening to guest lectures by Erwin Schrödinger and Max Born, and

attending parties with the likes of Fritz Zwicky and J. Robert Oppenheimer. "These were almost too interesting times", Richter recalled much later.

His comment might well serve as the motto for the place ever since, for Caltech attracted famous scientists the way its orange groves attracted fruitflies. Indeed, fruitflies landed on campus *en masse* when Thomas Hunt Morgan, the *Drosophila* king, arrived from Columbia University in 1928 bringing his 'fly room' with him. Five years later, in 1933, Morgan won a Nobel prize for his work in genetics.

Caltech has had no shortage of Nobel prizewinners on the staff; the school's greatest strengths, though, have not been in the life sciences, but in such hard sciences as rocketry, aerospace technology and physics. In the 1930s, Theodore von Kármán, the Hungarian physicist, arrived from Germany to direct GALCIT, the Guggenheim Graduate School of Aeronautics. Later, during the Second World War, the school's rocket programme developed a succession of inventions: the proximity fuse, JATO (jet-assisted take-off) and missiles of various types. Later still, JPL, Caltech's Jet Propulsion Laboratory, played a starring role in the US space programme.

The school itself was started by Amos Gager Throop, a man who had made, lost and then remade several fortunes in lumber and real estate by the time he became mayor of Pasadena. In 1891, Throop founded a little college there with a faculty of six, modestly naming the whole after himself: Throop University. Then, through a succession of metamorphoses duly recounted in the book, Throop University over the next three decades became Caltech.

Millikan was head of the place for 24 years. Ironically, the story of Millikan's life as a scientist is not one of the better told in the book. Early on, he had determined the value of Planck's constant and had verified Einstein's photoelectric equations, but just how he accomplished these things, and what they meant to physics, are not explained by the author. And although Millikan's best-known contribution to science, the oil-drop experiment — from which he deduced the charge on individual electrons by watching oil droplets float around in a chamber of air —

is arguably one of the more spectacular feats of experimental science, Goodstein reduces her account of this work to a single and rather elliptical sentence.

Some other Caltech luminaries receive



Relatively easy — Einstein visited Caltech in the 1930s and attracted much public attention.

similar short shrift. Murray Gell-Mann is mentioned only once, and Richard Feynman twice, but perhaps Goodstein felt that their careers are already too well known to warrant another telling.

If anyone is the star of *Millikan's School*, it is George Ellery Hale, a name familiar to all who have beheld astronomical photographs tagged with the credit line "Hale Observatories". Whereas Millikan was ever the stingy one, always trying to save a nickel or two (he "liked to boast that southern California's sunshine equalled several thousand dollars in salary"), it was Hale who went out and got millions of dollars for the construction of the observatory at Mount Wilson and later of that at Mount Palomar. Always a frail specimen physically, Hale suffered from a variety of health problems, and in 1923, after a succession of nervous breakdowns, he gave up the directorship of Mount Wilson Observatory. He decided at the time that he had "not the remotest intention of undertaking the organization of another institution of this kind", but he was soon plotting an even grander venture.

Hale's 200-inch telescope was a heroic project that took 20 years to complete. Sites were examined all over southern California, and more than a thousand sketches were made. Roads were put in, and a dome constructed. And then there was the making of the 200-inch disk



Turning the wheels — Millikan and his wife, Greta, setting out for the Harz Mountains in Germany in 1912.

itself: "Pouring the molten glass into the mold took seven hours; the disk was then sealed in the annealer, where it remained, cooling slowly, for many months." Grinding the mirror, which removed more than five tons of glass while exposing many slight flaws, took several more years.

The story of Mount Palomar Observatory makes fascinating reading, livened up as it is by one of those monumental battles of egos that often accompanies the doing of great deeds. Here, the conflict was between Hale and the president of the Carnegie Institution, John Merriam, who was vying for ownership of the telescope.

Goodstein, Caltech's archivist for 20 years, has produced a cleanly written, scientifically well informed account of

one of the world's foremost institutions for science and technology. Whether reading about Linus Pauling and the nature of the chemical bond, Charles Lauritsen's work with high-energy X rays, or relishing the unintentionally amusing boasts of the institute's former presidents ("There are no good appointments at Caltech — only superb ones. Of course, occasional mistakes occur . . ."), one will come away convinced that, with its 21 Nobel prizewinners and its 1,800 students bearing near-perfect entrance-test scores, this was and is one of the chosen Valhallas of latter-day science. □

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Missing the biotech boat

Alfred E. Middleton

The Biotechnologists. By Stephanie Jones. Macmillan: 1992. Pp. 252. £25.

How to describe *The Biotechnologists*? An impressionistic oral history of the biotechnology business? A primer for those considering starting up and running a biotech company? A comparison of the similarities and differences between biotechnology in the United States, Europe and Japan?

Any one of these is a more apt depiction of the essence of this book than the author's full-blown aim: "the analysis of the progress of biotechnology start-up companies in the USA and Europe through the perspective of the biotechnologists who founded them and/or now run them." To capture this perspective, Jones interviewed ten biotechnology chief executives and five venture capitalists in the United Kingdom and Europe, and nine biotechnology chief executives and one venture capitalist in the United States. All the US chief executives she interviewed were from so-called 'first generation' companies founded between 1978 and 1982, that is, from companies far removed from their start-up stages.

Jones has divided her book into three disparate sections: a preface in which she attempts to outline rules and models for the stages of a biotechnology company's growth; an introduction in which she competently relates the history of commercial biotechnology, particularly in the United Kingdom, and compares biotechnology in various countries; and the core, the interviews.

One large and several smaller mistakes compromise the effectiveness of

the book. Jones conducted her interviews in the United Kingdom and Europe during the winter of 1989–90, and in the United States during the summer of 1990. From then to the book's publication in January 1992, she committed a blunder of grievous proportions by impersonating Rip Van Winkle, slumbering through 1991 and completely ignoring what was happening last year in US biotechnology.

Last year was a watershed period in US biotechnology. Amgen became the industry's leader, doubling its revenues to \$699 million and dramatically increasing its profits — by 2,400 per cent — on the strength of sales of its two drugs, Epogen (EPO) and Neupogen (G-CSF). Starting in March 1991, 39 biotechnology companies floated initial public offerings, raising a total of \$1,200 million or an average of more than \$30 million apiece. Of these companies, about 21 were less than 3 years old, nullifying Jones's statement that "after 5 to 7 years, or perhaps much longer, a biotech enterprise may be ready to go public". In some cases, the initial public offerings were so well received and share prices maintained so well that these newly public companies were able to return to the public markets in a few months with second issues. A total of 88 separate US biotechnology stock issues were consummated in 1991, garnering more than \$4,000 million. Several of the US biotechnology companies whose chief executives Jones interviewed, raised additional capital through public offerings (Genzyme, \$142.9 million; Repligen, \$25 million).

Genetics Institute, whose chief executive, Gabriel Schmergel, was interviewed by Jones, lost a patent dispute to Amgen in March 1991 and was the target of a two-tier takeover offer by American Home Products for 60 per cent of its stock in November. Chiron acquired Cetus during the summer, creating a

combined company of 1,400 people and a launching pad for further growth. All these events strengthened US biotechnology, created formidable competition for biotechnology companies in other countries and put pressure on companies such as British Biotechnology to increase their capital base; yet Jones does not acknowledge them even in an epilogue.

While duplicating a section of Robert Teitleman's *Gene Dreams* (Basic Books, 1989; see *Nature* 342, 25; 1989) and being influenced by his pessimistic tone, Jones fails to pick up on the strongest feature of that book — the incisive personality and character descriptions. In her hands, the executives and venture capitalists are ciphers. We know them only by their words, not hers.

Consider the description in *Gene Dreams* of Robert Nowinski, one of the founders of Genetic Systems: "a strong, aggressive personality; a quick wit, boundless confidence — capable of thinking in large conceptual terms, an able fund raiser, quick to organize a team to follow his lead". You get a sense of a formidable presence. Nowinski was one of the three co-founders of Icos, along with George Rathmann, former chairman of Amgen, whom Jones interviewed. Rathmann comes across in *The Biotechnologists* as a practical, common-sense individual. His advice on funding: "get as much money as you can — go public when the time is right". Last September, Rathmann ousted Nowinski, the company's largest shareholder, from Icos. As well as his practical mind, Rathmann must have very sharp elbows indeed, but you would not know it from Jones.

Jones turns on her tape recorder and the interviewees ruminate. She makes no attempt to steer the monologues to critical issues — pricing, patents, government regulation. Those making the most outrageous statements stand out. Jim Vincent of Biogen wins for his comments on the superiority of US business, science and management. Herbert Schöemaker of Centocor is a close runner-up with his statement, "in a recent wave of forming start-ups, 1,200 new biotech companies have been formed". Thoughtful, perceptive statements such as those of Keith McCullach of British Biotechnology get lost in the shuffle.

As an oral impressionistic history of biotechnology, *The Biotechnologists* passes muster. To paraphrase John Dalton a century earlier, this book will no doubt be found interesting by those who take an interest in it. □

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Models for technology

Willem Hackmann

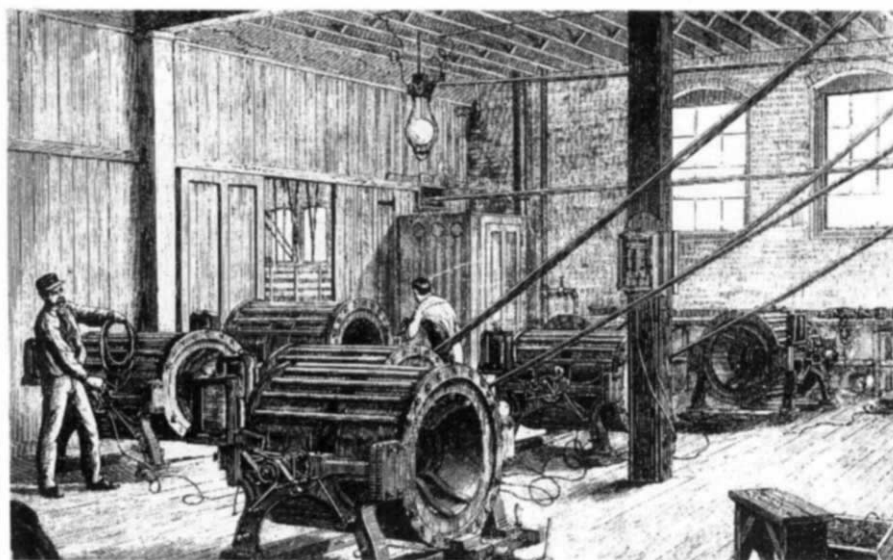
Innovation as a Social Process: Elihu Thomson and the Rise of General Electric, 1870–1900. By W. Bernard Carlson. *Cambridge University Press: 1991. Pp. 377. \$44.50, £35.*

At first glance there are many parallels between the childhood and early adulthood of Elihu Thomson and those other heroic figures of American technology, Thomas Edison and Henry Ford. Like them, he came from a humble background (his parents were Scottish working-class immigrants) and he was forced to curtail his education in order to help support his family; but thanks to plenty of ambition and hard work, he overcame these limitations and became a successful inventor. Unlike Ford, he grew up in the heart of a bustling industrial city, Philadelphia, and unlike Edison, he received a thorough early education in science. Both of these aspects are considered to be significant by Bernard Carlson, who interweaves Thomson's personal history into the social and technical milieu of his times.

Thomson conformed to those other hallmarks of the late nineteenth-century technological innovator, having a passionate interest in mechanical devices and a way of learning about science through the building of such machines (an activity that according to Carlson leads to 'craft knowledge'). Indeed, a central theme of this book is the rise of late nineteenth-century technology through the interplay of scientific and craft knowledge. (It is a moot point whether this interplay is relevant in modern research and development.) Carlson attempts to establish the factors that made Thomson's activity so successful in both technological and business terms from 1870 to 1900. That key period began with Thomson's graduation from the Philadelphia Central High School and the start of his career as a chemical analyst in an ironmaster's laboratory, and ended when the General Electric Company established its Research Laboratory and Thomson relinquished his position as chief innovator of the company. The phenomenal growth in American technology during this period is reflected in Thomson's life; he and the electrical industry grew and matured together.

With Edwin J. Houston, a teacher at the Central High School, Thomson established the Thomson-Houston Electric Light Company in Philadelphia to manufacture the arc-lighting system that they had invented. In 1880, a new company was formed, the American Electric Company in New Britain, Connecticut, later renamed the Thomson-Houston

Electric Company of Lynn, Massachusetts. In contrast to the New Britain venture, the Lynn enterprise was a great success, largely owing to the vision of Charles A. Coffin, the vice-president of Thomson-Houston. Coffin's experience in the expanding shoe-industry of the 1870s had taught him important lessons that he could now apply to the rapidly changing electrical manufacturing industry. Initially, small arc-light systems were



Dynamo-testing room in the Thomson-Houston factory at Lynn, Massachusetts (1885).

sold to individual users, but Coffin appreciated that the key to unlocking an enormous potential market for electric lighting was to promote central stations to be run by public utility companies. The new alternating-current technology that had been developed by Thomson, among others, made this concept possible. Thus, first Coffin learned how to create and sustain a large market for his product, and second he discovered that technology was not a static, but rather a dynamic, component of business strategy. Technology allowed costs to be reduced and could be used to capture new markets. The Thomson-Houston Company was successful in the electric-lighting field because the firm managed to integrate technology, marketing strategy and business organization. These were also the years in which Thomson was at his most productive as an inventor. In 1892, the company merged with the Edison General Electric Company to form that powerful conglomerate, the General Electric Company. Thomson refused a directorship,

but he continued to head product development in his "Model Room".

In addition to his initial dynamo (generator) and arc-light developments, Thomson invented an alternating-current motor, the first high-frequency generator and high-frequency transformer, the three-coil generator, electric welding by the incandescent method, a watt-hour meter, and an X-ray system with a double-focus tube. The third most prolific inventor in American history, his 696 patents have been surpassed in number only by Edison with 1,093 and by the railroad pioneer John O'Connor with 949. Thomson died in 1937, investigating scientific problems almost to the end.

Carlson has examined the broader issues of technical innovation as a social process by taking the life of Thomson as

a case study. Although there are instances where one wonders whether the author has been justified in describing certain specific events in Thomson's life in terms of this general theme, the approach gives useful insights into the relationship between innovation and the extraordinary rise of technologically based business in late nineteenth-century America. As Carlson points out, innovation is the synthesis of creative acts in the technical, organizational and marketing realms. Thomson needed a business entrepreneur such as Coffin to turn his inventing skills into a commercial success. The period ends with the founding of General Electric's Industrial Research Laboratory; innovation was by then a corporate activity. The pressures of the market place made it no longer possible for an inventor such as Thomson to work alone in his "Model Room". □

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The temporal enigma, again

Huw Price

The Mind of God: The Scientific Basis for a Rational World. By Paul Davies. Simon and Schuster: 1992. Pp. 254. \$22, £16.99.

The Matter Myth: Dramatic Discoveries that Challenge our Understanding of Physical Reality. By Paul Davies and John Gribbin. Simon and Schuster/Viking: 1992. Pp. 320. \$12 (pbk), £16.99 (hbk).

The Mind's Sky: Human Intelligence in a Cosmic Context. By Timothy Ferris. Bantam: 1992. Pp. 281. \$22.50, £16.99.

The Capricious Cosmos: Universe Beyond Law. By Joe Rosen. Macmillan, Inc. (USA): 1992. Pp. 224. \$19.95.

PAUL Davies presents us with a temporal enigma. Twenty books in as many years, a successful career as a 'serious' physicist, and a very active round of broadcasts and public lectures — how on earth does he find the time? Whatever his secret, it works as well in Australia as it used to in the United Kingdom. Davies has become a frequent contributor to the scientific side of Australian cultural life in the past couple of years, and a very popular one: his most recent public appearance at the University of Sydney filled three or four large lecture theatres.

In his twentieth book, *The Mind of God*, Davies tackles some of the traditional Big Questions that generate this public interest, and some interesting new ones: Can science explain everything? Why is it that science can explain anything, and what does this tell us about our relation to the Universe? Does the existence of the Universe need any external cause or creator? Could it have been otherwise? Is it a giant computer? It is not easy to write intelligently and accessibly about issues at this level, but Davies here does it exceptionally well. As a whole, the book is an impressive and rather moving reflection on physics, humanity and the Universe, as it appears to one of the discipline's most able communicators towards the end of the twentieth century.

Let me mention three points of detail that left me unsatisfied. First, the suggestion that physical systems might be computers calculating their own behaviour seems uncomfortably close to the idea that the world is a language describing its own state (as it would be for the professors of Swift's Academy of Lagado, for example, who proposed eliminating words in favour of the things themselves). Does the Solar System



Humans have hunted whales for around a thousand years, killing millions of them for their valuable meat, oil and bones. These illustrations are just a sample from the many more that appear in *Men and Whales* by Richard Ellis (Robert Hale, price £25), the "first comprehensive history of the whale's turbulent — and always controversial — relationship with humankind".

really compute its planetary orbits in any more interesting sense than a cat on a mat describes its own location?

Second, the question of why the Universe is comprehensible at all — why it is not a *P2C2E*, in Rushdie's useful technical notation* — seems to me more slippery than Davies allows. How do we know that the Universe is significantly comprehensible? What would it look like if it wasn't, by and large? Could we tell?

Third, the book's concern with the question of whether the Universe requires a creator to get it started seems to me a little laboured. Davies describes the Augustinian viewpoint, which neatly sidesteps our usual obsession with *beginnings* by placing the creator outside time. If modern cosmology tells us anything it is that this is the right move: the idea that the Universe needs to be externally started has no more objective basis than the idea that it needs to be externally stopped. After all, if there were a place for a non-Augustinian creator, there would also be a place for the question of at which end He started: did He create the Universe at the Big Bang and let it run forward, or create it at the Big Crunch and let it run back? Cosmology does not take the latter possibility

seriously, and neither should it the former.

Far from detracting from the book, however, points of this kind illustrate one of its virtues. Despite its scope and accessibility, it is sufficiently thorough to permit critical engagement about points of detail. *The Mind of God* would thus make an excellent basis for an undergraduate philosophy course on Science and the Big Questions, and may also be warmly recommended to the general reader.

I found *The Matter Myth* less satisfying. Here, Davies and John Gribbin claim to describe a revolutionary change taking place as a result of recent developments in science: the overthrow of materialism, and liberation from the alienation and depersonalization that has so long accompanied it. It turns out that 'recent' is being used rather liberally: two of the key ingredients of this claimed revolution are our old friends relativity and quantum mechanics. It has long been commonplace that relativity views the Universe as a 'static' structure incorporating time, rather than as a dynamic entity changing in time; and that quantum mechanics leads to indeterminism. More importantly, it has long been obvious that there is no comfort for humanism in any of this. Quantum indeterminism does nothing for our free

*"A Process Too Complicated To Explain"; see Haroun and the Sea of Stories, page 57, Granta, 1990.

will; while what could be more impersonal than the block Universe, in which our entire existence is some almost insignificant thread?

Indeed, it seems to me that the main thesis of *The Matter Myth* rests on an ambiguity in the notions of materialism and mechanism. These terms are sometimes used in contrast to humanism or idealism, and sometimes with reference to a specific (say, newtonian) conception of the fundamental nature of the world. These two senses are somewhat mixed up when Davies and Gribbin describe mechanism as "the belief that the physical Universe is nothing but a collection of material particles in interaction, a gigantic purposeless machine, of which the human body and brain are unimportant and insignificant parts". Provided that we keep the senses distinct, it is possible to see that a world view may be materialist in the first sense — that is, impersonal — without being newtonian. And that, surely, is what the physics of the past century has given us. (Lest it be suggested that I have ignored genuinely recent developments concerning chaos and nonlinearity, what comfort is it if it turns out that we and our Universe are *unpredictable* machines? Is a rogue robot any more human than its predictably programmed cousin?)

Leaving aside its claims concerning materialism, there is a lot to recommend the book. Davies and Gribbin are both masters of the art of describing complex scientific ideas to lay audiences, and in this respect they maintain their usual high standards. So read it for this, but take the news of the revolution with a pinch of salt.

One of the great challenges of the popular science genre is to be entertaining as well as intellectually stimulating. A writer who does well in this respect is Timothy Ferris, whose *The Mind's Sky* is an enjoyable ramble through a variety of topics to do with minds, brains and their place in the cosmos. I particularly liked the suggestion that there might already be a self-extending information network, spreading the accumulated knowledge of diverse civilizations through the galaxy. Let us hope that when we find our local terminal it is in working order. (How frustrating to have to wait 10,000 years for the Repair-Creature.)

By contrast, *The Capricious Cosmos* is a salutary example of the dangers of venturing into print beyond one's field. The basic guidelines here are those of foreign travel: try to pick up a smattering of the language, and tread lightly. This book is a physicist's journey into metaphysics, but fails on both counts. □

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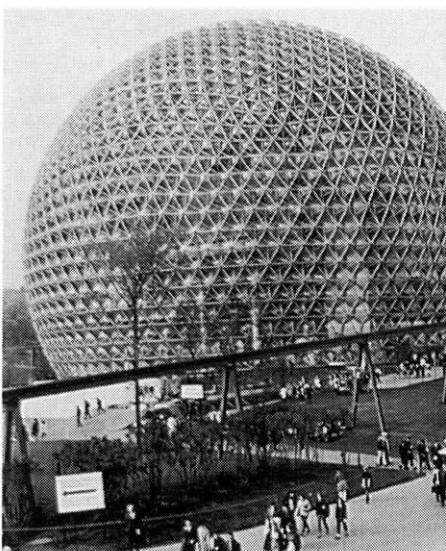
From brilliance to crackpottery

Ian Stewart

Cosmography: A Posthumous Scenario for the Future of Humanity. By R. Buckminster Fuller (adjuvant: Kiyoshi Kuro-miya). Macmillan, Inc. (USA): 1992. Pp. 277. \$24.95.

AT a time when a 60-atom carbon cage named in honour of an architect has boldly been hailed by *Science* as "molecule of the year", a book on the working philosophy of the man himself would seem only appropriate. That the molecule should be called "buckminsterfullerene" rather than 'truncatedicosahedrene' reflects the extent to which Buckminster Fuller succeeded in putting his ideas across to humanity. But not every innovation for which he tends to be given credit is as original as his admirers imagine.

In 1983, on the day that Buckminster Fuller died, the manuscript of *Cosmography* was found stacked in the middle

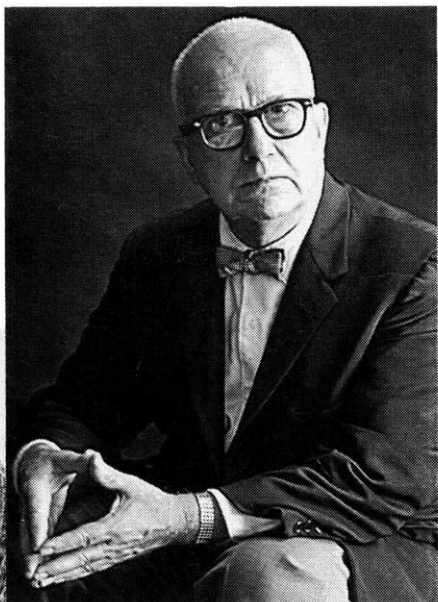


of his desk. Attached to it was a note to his daughter and grandchildren, stressing "the extraordinary importance of my now being written book". Kiyoshi Kuro-miya, the "adjuvant", a term that Fuller borrowed from medicine, has sensibly preserved the author's "idiosyncratic concepts, tone, syntax, and phraseology". As a result, we have a book that lets its readers get inside that extraordinary — and rather frustrating — mind.

Fuller saw the human race as still living in the Dark Ages, locked into a futile circle of misinformation, perverted by big business, militarism and organized religion. He sought the rebirth of humanity in a better future, a world in which individual genius would be nurtured rather than suppressed. This

philosophy led him to conduct his life as an "experiment in individual initiative". It led to some remarkable achievements, most notably the invention of the geodesic dome. It led him to formulate the idea of "synergetics", in which objects are replaced by systems, balanced between their internal and external features. And it led him to make scathing but rather generalized criticism of conventional science. All of this was laced with a characteristic obsession with numerology and an uncompromising view of the importance of truth.

Cosmography reveals the astonishing strengths of Fuller's mind, as well as its flaws. One cannot doubt the sincerity of his vision of the human predicament, nor



Science Photo Library

Buckminster Fuller and the glass geodesic dome that he designed for the US pavilion at EXPO '67 in Montreal, Canada.

fault many of his insights: "Big money, big religion, and big politics are all still deliberately frustrating human comprehension"; or, as my father once said to me, "they teach you enough to take orders, but not to give them." Equally, it is hard to agree that *every* child is born a genius. With more than 200,000 geodesic domes in existence, one can hardly quibble over Fuller's success and originality as an architect. But what about his claim that stacks of cubical boxes tend to separate as they grow higher because local verticals are not parallel on a spherical Earth? To Fuller this is important, representing yet another nail in the coffin of the "misinformed XYZ" coordinate system beloved by physicists. To me, and to physicists, it shows that Fuller can't do a back-of-the-envelope calculation. Except that he can — when he wants to. His geometric ingenuity is striking. Who else would have noticed that you can fold up

four circles and assemble them to form a cuboctahedron (what he calls "vector equilibrium")? On the other hand, who else would see a cryptic, mystic message in such a possibility?

Far sadder is his tendency towards numerology, and the belief that on such a basis he could think his way towards an understanding of the fundamental structure of the Universe. "This 28 we multiply by the twoness of internal mite rearrangeability of the mite's 2 A and 1 B modules, giving us 56 arrangements of the same total energies inter-energy-proclivities of each coupler". After pages like this we are told that "synergetics provides. . . a more sophisticated understanding of subatomics than that of the nuclear physicist whose favourite tool is the atom-smasher."

It is this kind of lurch from brilliance to crackpottery that makes Fuller so infuriating. He is far more convincing

when he avoids specifics. The defects of his numerology are obvious, but he still has a valid viewpoint when he suggests that smashing atoms to bits may not be the cleverest way to decide how they work. Reductionist biologists, just as obsessed with the DNA code as is Fuller with his numerology, would do well to heed his warning that "nature invents many alternative circuits that provide the same results"; and to take on board the principle that outsides matter just as much as insides. And we should all think long and hard about the failures of "traditional human power structures and their reign of darkness". *Cosmography* builds on too many of Fuller's flaws to be a great book; but its author, flaws notwithstanding, was a great man. His book lays bare his own enigma. □

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Twinkle, twinkle

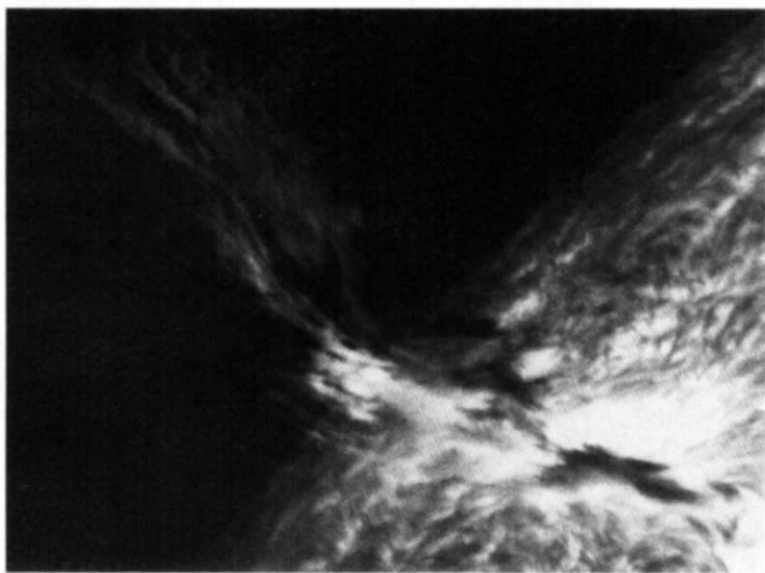
David W. Hughes

Stars. By James B. Kaler. *Scientific American Library*/W. H. Freeman: 1992. Pp. 274. \$32.95, £17.95.

THERE are three great problems when it comes to studying stars. Most stars are a long way off and, with the exception of the Sun, they are perceived as little more than points of light. So we know the detailed vagaries and variabilities of only one stellar surface, and that has been studied for only a couple of centuries. Stars are huge turbulent spinning spheres of glowing gas energized by intense nuclear reactions in their deep interiors. But the radiation that we can detect comes only from an outer layer a thousand or so kilometres thick. The remaining 99 per cent of the stellar volume is beyond our ken and can be probed only by the theoretical extrapolation of physical equations. And the pace of stellar evolution is so slow that astronomers rarely see an individual star age. Our understanding of the life-cycle of a star has to come from glimpsing a host of different ones during their baby, youthful, middle-aged and geriatric periods.

Coupled with these stellar problems are a series of traps set for people who write about the stars, traps that seem to

originate from the time-honoured and rather dreary order in which most astronomy textbooks approach the subject. James Kaler unfortunately falls headlong into these traps. The first 60 pages of his book regale the reader with risings and settings, celestial poles and meridians and telescopic mirrors and detector designs. Only after ploughing through this overly long introduction does one start to get some feeling for what a star actually is. But even then the true picture has to await a discussion



A powerful solar flare, the result of an intensely hot electromagnetic explosion in the corona, produces vast quantities of X-rays which brighten the chromospheric gases.

of black-body radiation and atomic spectroscopy. At last, by about page 85, the reader begins to realize that stars are not all the same but have luminosities that range from a million times brighter than the Sun to a million times fainter, masses that range from 120 to 1/13 solar masses, and sizes that range from a few kilometres to the size of

the orbit of Saturn.

I greatly enjoyed the section of the book dedicated to stellar interiors, and Kaler has done a first-rate job of explaining the complexities of stellar energy generation and transportation. But I was a bit surprised that he then proceeded to tell how the Sun would evolve in the future (giant, helium flash, supergiant, planetary nebula, white dwarf and so on) before he revealed how the Sun actually came to be what it is today. I also thought that the general reader would get slightly confused by his brief explanation of the Sun's journey along the Red Giant Branch and the Asymptotic Giant Branch of the Hertzsprung-Russell diagram.

Balancing those portions of the book that I would have omitted were sections that I would have expanded. I would have liked more about why the luminosity function has the form that it has, why only half the stars are binaries and how planetary formation is related to star formation?

Even though stars are the primary source of energy in the Universe, and our planet and all living things are made of stardust, I still found Kaler's statement that "to know ourselves we must know the stars" a touch pretentious. I also found my mental imagery linguistically distorted when I read that "stars come dripping from the fonts of interstellar space" and that "as they age they pump enriched matter back into the wallsprings of creation"; but after a time you get used to it. And Kaler can be forgiven, because it is clear that stars are his great love.

His enthusiasm becomes infectious. I was even able to overlook the fact that he misspelt the christian name of my hero Edmond Halley; but to state that Halley went to Cambridge rather than Oxford was going too far. I was heartened by the many times that Kaler admits that astronomers are still mystified by many aspects of stars and stellar evolution. The true joy of working at the frontier of astronomy is portrayed very well.

Stars abounds with beautiful stellar pictures and, as one would expect from a book from the *Scientific American* stable, the standard of the illustrations and figures is first class. □

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The mushrooming brain

Ryan J. Huxtable

Food of the Gods: The Search for the Original Tree of Knowledge. By Terence McKenna. Bantam: 1992. Pp. 300. \$21.50.

In the beginning were the great savannahs of Africa, on which presapient hominids, with brains one-third the size of ours, eked out marginal existences as gatherers and inefficient hunters. From the excrement of grazing ruminants grew mushrooms, *Stropharia cubensis*, containing the hallucinogen psilocybin, which pre-man sampled. And lo! Visions came onto him, and self-consciousness, and speech. The increased insight allowed by the "chemical binoculars" of the mushroom made for more successful hunting and triggered a rapid increase in brain size. Thus from out of the mushroom was born man. The sexual arousal caused by psilocybin allowed him to reproduce better, to the detriment and extinction of the other non-turd-examining hominids. And man lived at peace in the world.

But woman learned to grow plants and embraced a sedentary life in her garden. She no longer ate the mushrooms, because the hallucinations made her oversleep in the morning instead of hoeing the fields. And thus began the great retreat from the natural world, which brought all the evils of history on mankind. And the ice age ended, and the glaciers marched north from Eurasia, bringing desiccation to the grasslands of Africa. So man was forced east of Eden — into Asia — where there was a dearth of mushrooms. This drove the continued evolution of language, leading to the emergence of the ego, from whence comes evil. Or so according to Terence McKenna.

For proper comprehension, *Food of the Gods* must be viewed not as science, or even sociobiology, but as a creation story, an exercise in reshaping old myths for modern society. It is a retelling of the Fall of man, and original sin, for the Fall came from the same causes that allowed our evolution from proto-humans. In essence, it is a morality tale, the author himself using the phrase, "a pharmacological pilgrim's progress". So, for all the pseudoscientific trappings, perhaps the arguments in this book are no more to be taken literally than any other creation story. If anything, *Food of the Gods* is poetry disguised in the language of

science. Indeed, we are treated to a dithyramb on the Joycean pleasure of botanical descriptions: "Leaves . . . sessile, linear-lanceolate or lanceolate, acute or acuminate . . .".

So McKenna would have us believe that all came from the mushroom. The radical nature of his argument is perhaps shown by his describing the inventor of



mycolatry, R. Gordon Wasson, as conservative in his views on fungi. Simplistic as it may seem, the book is thought-provoking. The mushroom gave us, says McKenna, self-awareness, religion, a thin skull, a large brain and language. Man ate the mushroom and learned to speak. Language is the only reality; reality is produced by language. Yet, inconsistently, this good deconstructionist goes on to say that "the psilocybin intoxication is a rapture whose breadth and depth is the despair of prose". But no matter. When a whole universe is compressed into the symbolic notations of 300 pages, one must expect a few inconsistencies. The main effect of psilocybin was in the domain of language. It "excites vocalization, empowers articulation, and transmutes language into something that is visibly beheld." In other words, if reality is language, and hallucination is language, then reality is hallucination. There is no reflecting mirror, no seeing through a glass darkly, no

material stone for Samuel Johnson to kick in refutation of nonexistence. Life is but a dream.

Language also makes us an epigenic species, a species whose future is determined not by genes but by culture, which in turn is determined by language. McKenna believes that language was created by the mutagenic action of hallucinogens on brain organelles involved in the processing of signals. Opening the doors of perception with drugs allowed the escape of utterance.

Africa has few hallucinogenic plants, and this, the author thinks, is the result of the fall of man: "Can it be mere coincidence that the longer an environment has been exposed to human beings, the fewer its native hallucinogens?" The apple, once eaten, is gone; a foretaste of the desolation that man has visited on the globe, and will continue to visit, unless he returns to his origins in the archaic period of 7,000–10,000 years ago, back to the turds and mushrooms and shamans, back to the strong silent hunt, alone with the mushroom visions and the wilderness, away from television and the seductive gossips around the vegetable patch.

Another two cents worth is added to the perennial soma controversy. Soma is the hallucinogenic preparation of the old Ayurvedic scripts of India that conferred immortality on its consumers. The source of soma has been variously identified as *Ephedra*, *Cannabis*, *Peganum* and *Amanita* (a mushroom), as well as a herbarium of other plants. Here it is equated with our old friend *Stropharia cubensis*. Wasson's identification of soma as *Amanita muscaria* is challenged by McKenna on the grounds, among others, that, unlike *S. cubensis*, *A. muscaria* does not grow on cow excrement. Indeed, he says, *S. cubensis* is thought to have originated in Thailand, which raises the unanswered question of why man, thrown out of Africa, ran out of mushrooms.

Accuracy of detail seems unimportant to such a vision. In correction of some of the numerous mistatements in the book, the cactus genus *Lophophora* does not include Sonora in its range, but comes from Chihuahua and parts of Texas; not all steroids are alkaloids; a substance found naturally in the body can still be a drug; and coffee was first used in Europe not in 1100 but around 1500, in Venice.

Upon a peak in Darien, the author gazes at the whole of human experience with a wild surmise. Judgements come

thick, fast and sweeping, couched in the adjectival prose used by that moiety of North America not addicted to compound nouns: "Medieval Europe was one of the most constipated, neurotic, and women-hating societies ever to exist . . . ruled by gouty, beef-eating men wearing dresses." Or how about: "The grandeur of Rome was the grandeur of a pig sty masquerading as a military brothel"? Well, yes. Such statements are as impossible to disprove as they are to prove. For all its colour, such language is devoid of meaning. So again we conclude that this is an excursion into the

realm of morality rather than knowledge. It is a peculiarly American activity, this construction of manichaeon universes balanced on conjecture and black boxes. Such holy simplicities are not for the cynical who can conceive of complexities having more than a unitary origin. But it is the simplicities of this book that provide both its moral force and intellectual weakness. □

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Metamorphosis

John C. Marshall

Pride and a Daily Marathon. By Jonathan Cole. Duckworth: 1991. Pp. 194. £14.99.

WHEN Gregor Samsa awoke one morning, he found himself unable to get out of bed; he could not control the movement of his limbs and, without vision, was even unsure of the position of his body: "When finally, in a kind of rage, he summoned all his strength and recklessly thrust himself forward he had mistaken the direction, striking the lower bedpost a violent blow, and the sharp pain he felt informed him that it was this lower part of his body that was perhaps the most sensitive at the moment." Kafka's man had metamorphosed into a giant insect.

The transformation that befell Ian Waterman in 1971 was scarcely less horrifying. As a perfectly normal young man of nineteen, a skilled butcher, he took a new job in the island of Jersey, in the English Channel. The boss, impressed by his speed, accuracy and dedication, was about to offer him a junior partnership in the business. Then fate struck. After what initially seemed no worse than a bout of gastric flu, Waterman, like Samsa, found that he was incapable of controlling his body. In *Pride and a Daily Marathon*, the sympathetic neurophysiologist who befriended and helped him documents Waterman's progress over the next 20 years.

I write 'helped' rather than 'treated' because Waterman's condition was totally intractable. He had suffered a neuropathy of the peripheral nervous system that deprived him of the ability to feel touch and of all proprioceptive and kinaesthetic sensation below the neck. Such information is coded by myelinated nerve fibres with a relatively large diameter and conduction velocity. It was these nerves that had been functionally destroyed by an inflammatory immune

reaction to an (unknown) infective agent.

Some sensations, mediated by smaller, slower, unmyelinated nerve fibres, remained: pain, temperature, deep pressure and muscle 'effort'. There was no disorder of the motor nerves; the ability to move *per se* was retained, with normal strength. But this skill is of limited utility if you do not know where your body is to begin with, which direction it is moving in, and where it has arrived at during the course of movement. Loss of the 'sixth sense' of limb position, static or dynamic, seemed to condemn Waterman to 'life' in a wheelchair with a body that was not his own and over which he had no control.

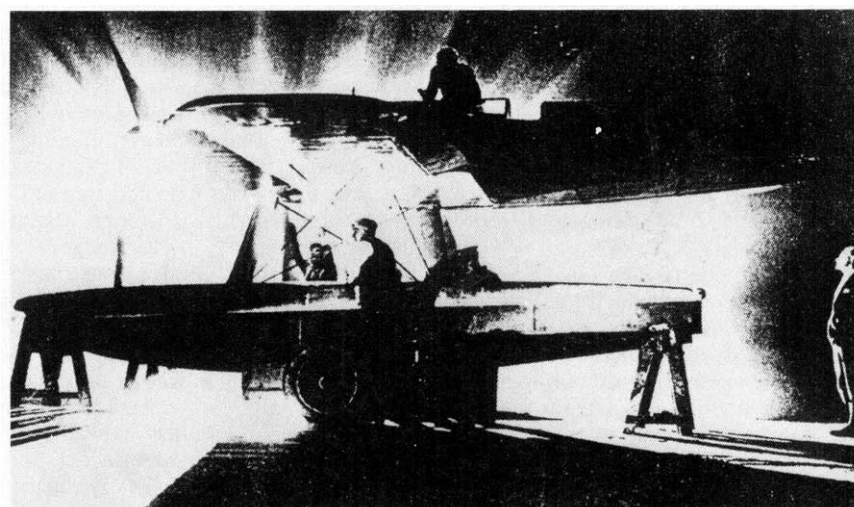
The story that Cole recounts is how Waterman, by sheer willpower, guts and persistence, learned to do almost everything that is normally achieved automatically by the inner senses. No physiological recovery has taken place, but Waterman now manages his body by visual feedback; he constantly monitors every movement by sight. What he can do in this fashion is nothing less than astounding.

To most intents and purposes Waterman leads a normal working life, although to call it 'normal' is something of an insult to the supernormal effort and concentration that he requires to do anything. For a man who can collapse in a heap on the floor if he sneezes (and hence loses, albeit momentarily, visual contact with himself), the description of life as "a daily marathon" is a gross understatement.

Cole's book (which is really coauthored by Waterman himself) is a model of popular science writing. An intimate biography of Waterman is interwoven with sufficient anatomical and physiological information for the general reader to understand the basic neurology of the peripheral nerves and their disorders. During his rehabilitation, some members of the medical and paramedical professions were very helpful, whereas others showed little understanding of his efforts. But, unlike the Samsa household, Waterman's friends and family were a tower of strength. (Connoisseurs of coincidence will note that Waterman's mother is called Felice.) Above all, the story is a tribute to what Waterman describes as his "pride, bloody pride". □

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■ Extending the tradition of writing about illness is *The Law of White Spaces* by Giorgio Pressburger, published last month by Granta. Part fact, part fiction, part autobiography, the book contains five beautifully crafted stories about doctors forced to confront "mysteries bigger than themselves". Price is £12.99. (To be published by Pantheon in this United States later this year.)



This aeroplane, designed by R. J. Mitchell (far right) at Supermarine, provides the clichéd image of English aviation, winning the world speed record in 1931 and being mistakenly portrayed as an early version of the Spitfire. In *England and the Aeroplane*, David Edgerton charts the history of English aviation, challenging current orthodoxy by arguing that England should be seen as a technological, industrial and militant nation. Published by Macmillan, £35 (hbk), £14.99 (pbk).

Games that people play

Martin Shubik

Prisoner's Dilemma: John von Neumann, Game Theory and the Puzzle of the Bomb. By William Poundstone. Doubleday: 1992. Pp 290. \$22.50.

WILLIAM Poundstone is a generally skilful science writer who in this instance has tried to write three worthwhile books in one. They are another decent biography of John von Neumann; a good popular account of the theory of games that is both balanced and accurate; and an analysis of the development of nuclear war strategy. Unfortunately, Poundstone has produced a *mélange* that does not do justice to any one of these topics.

Prisoner's Dilemma contains a well-written but slight biography of von Neumann. Apart from supplying some of the usual anecdotes and giving a sketch of the chronology of von Neumann's life, the author does little to explain this man's brilliance as an applied mathematician. Whether or not he was nice is of some popular interest, but as with Gauss, Newton, Einstein or Bohr, such detail is trivial in comparison with his intellectual output.

Game theory is also badly treated. Poundstone deeply misinterprets and misunderstands von Neumann's commitment to cooperative game theory that he and Oskar Morgenstern clearly spelled out in the first chapter of their book *The Theory of Games and Economic Behaviour* (1944). Von Neumann perhaps could best be described as conservative and hawkish; but the 'prisoner's dilemma' type of noncooperative game theory adopted by political scientists and discussed by Poundstone was utterly foreign to him, and to the best of my knowledge he never used it. (In a personal communication, von Neumann once told me that he had little use for noncooperative game theory. I was trying to persuade him that for some problems in economics the use of this theory might be the right approach, as it had been followed fruitfully in 1838 by Cournot, the father of mathematical economics.)

The seminal paper on game theory was von Neumann's 1928 article on two-person zero-sum games (where the gain of one participant is the loss of the other). As early as 1928, Morgenstern had noted the strategic dilemma of these games in a discussion of conflict between Sherlock Holmes and Moriarty. At the time, von Neumann and Morgenstern did not know each other, but later in Princeton they worked together to help

construct a theory of cooperative games. Both justified their emphasis on cooperative games because they felt that the correct scientific approach was to devise a new static equilibrium theory for the social sciences. They stated quite clearly that they thought it was far too early to offer a satisfactory dynamic theory and that it was possible that the structure of such a theory would differ considerably from the static theory.

Poundstone states that "Von Neumann and Morgenstern got sidetracked in their treatment of games of more than two persons. Their approach, while not wrong, no longer seems the most useful or most illuminating one." Apart from ignoring the large and still growing literature on the applications of cooperative game theory to voting, the pricing system, cost accounting and communication networks, this statement shows that the author is unaware of the important developments of techniques for studying many-person games. This is further illustrated when he writes, "Unfortunately, the complexity of games, and of the necessary computations, increases exponentially with the number of players. If the economy of the world can be modelled as a 5-billion-player 'game', that fact may be of little practical use." On the contrary, the development of methods to analyse games involving a continuum of agents or, for that matter, a countable infinity of players, are beginning to provide precisely the methods needed to study economics and politics.

It is difficult to sort out Poundstone's third purpose — to discuss US nuclear cold-war strategy in relation to the game theory proposed by von Neumann and Morgenstern. Clearly Poundstone does not understand von Neumann's caution in using mathematical models. Von Neumann's thoughts on nuclear war were influenced very little by formal game theory, and Poundstone is wrong in suggesting that von Neumann might have been a model for Dr Strangelove. Those involved in the use of simple cold-war noncooperative game theory were Herman Kahn, Daniel Ellsberg, Tom Schelling and, to some extent, Albert Wohlstetter and Henry Kissinger.

The innuendo throughout the book is that the highly imprecise (but imaginative) use of analogies, experiments and simulations based on the two-by-two matrix was somehow connected with the ideas and concepts of von Neumann. The reality, though, is that he had little if any use for this sort of theorizing. Among the key deep insights of von Neumann and Morgenstern was that, even if one made highly simplifying assumptions about bloodless, passionless, rational men, the attempt to extend the concept of rational behaviour beyond one individual is filled with

many difficulties and paradoxes.

The very title of Poundstone's book bespeaks of popular science. The prisoner's dilemma is easy to comprehend and offers an excellent way to mislead lay persons about the main contributions of game theory. It is a shame that an author with the writing talent of Poundstone did not bother to understand enough about game theory or von Neumann's ideas.

The book on von Neumann, Morgenstern and the development of the theory of games remains to be written. □

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Out on a limb

Michael C. Corballis

The Left-Hander Syndrome: The Causes and Consequences of Left-Handedness. By Stanley Coren. John Murray: 1992. Pp. 308. £17.95.

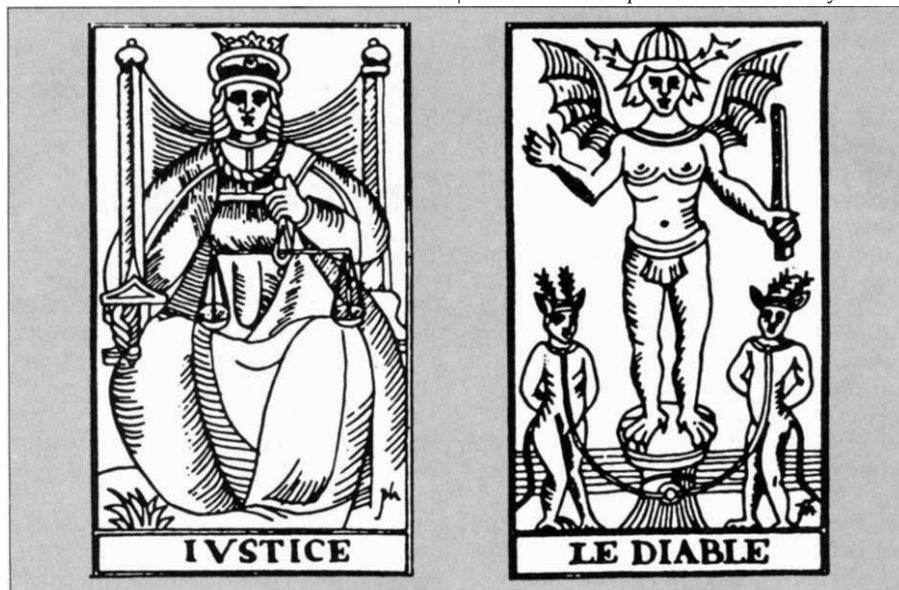
THERE have been many books written about handedness, and to those of us in the trade there is a touch of *déjà vu* in this latest offering by Stanley Coren. We read again the list of famous left-handers, the quotations from the Bible, the litany of derogatory terms referring to or derived from left-handedness, the myths and prejudices, ancient and modern, associated with both handedness and the two sides of the brain. But there is also much that is new here, and if nothing else this book is a marvellously full compendium of facts about left-handedness. It is written in an engaging, conversational style that will appeal even to those with only a casual interest in the topic.

Coren's main contributions to the study of handedness have been empirical, typically in the form of large-scale surveys of handedness, footedness, eyedness and earedness and the relationships between them, on handedness in families, and on handedness as depicted in works of art going back 5,000 years. Coren uses the facts and figures he has accumulated to test various theories of handedness and sidedness, and indeed to counter some of the more fanciful ones. His chapter on "Psycho-Neuro-Astrology" is a valuable debunking of the 'left brain/right brain' dichotomy that permeates popular folklore, and should be read by all magazine editors.

But the book will provoke its own share of controversy. Coren's main theme becomes apparent in the chapter that asks, "Are Left-handers Pathological?" He reviews evidence that left-handers are indeed over-represented

among virtually all of the pathological or 'deviant' groups that one might think of, including alcoholics, drug abusers, homosexuals, the depressed, the suicidal, the allergic, the brain damaged, criminals, epileptics, juvenile delinquents, the mentally retarded, even bed-wetters. (And men, one might add.) He finds evidence for the influential but controversial idea, originally proposed by the late Norman Geschwind of Harvard Medical School, that left-handedness is associated with autoim-

dered recent genetic models of handedness. In a chapter on the inheritance of handedness, he concludes that all humans are genotypically right-handed, implying that left-handedness must be a sign that something has gone wrong. But he ignores increasingly influential genetic theories, such as those of the British psychologists Marian Annett and I. C. McManus, that quite successfully model the variation in handedness and its inheritance. Later in the book, Coren does seem to accept that there may be a



Predicting fortunes — in the classic tarot cards, justice is right-handed whereas the devil is left-handed.

mune disorders, and that both result from a raised prenatal exposure to testosterone. He concludes this woeful inventory by reviewing two studies, carried out with Diane F. Halpern, that purportedly reveal left-handers to have a shorter life expectancy than right-handers — a claim that almost carried the seeds of its own destruction when irate left-handers threatened him and other right-handers with an early death.

All this begins to make one wonder if the age-old myths about the inferiority of left-handers are still with us. But Coren hastens to assure the reader that these various afflictions do not apply to all left-handers. He regards left-handedness as a "soft sign" that might alert physicians or teachers to the possibility of associated disorders, and coins the term "alinormal" (roughly, 'other than normal') to try to escape the negative connotations of 'abnormal'. Even so, I suspect that he may have gone a little too far out on a limb. There are some impressively large-scale studies that have failed to support the idea that left-handedness is due to brain pathology, and the most recent evaluations of Geschwind's rather baroque theory seem to weigh against it.

The pall over left-handedness might also have been lifted had Coren consi-

dered recent genetic models of handedness. In a chapter on the inheritance of handedness, he concludes that all humans are genotypically right-handed, implying that left-handedness must be a sign that something has gone wrong. But he ignores increasingly influential genetic theories, such as those of the British psychologists Marian Annett and I. C. McManus, that quite successfully model the variation in handedness and its inheritance. Later in the book, Coren does seem to accept that there may be a

genetic condition that makes some people naturally left-handed; here he makes the valuable point that a switch in handedness owing to some pathology would result in more pathological left-handers than pathological right-handers, as there are many more natural right-handers to begin with. Towards the end of the book, Coren changes tack and suggests that at least some of the woes of left-handers, including their reduced life-expectancy, may be due to the ways in which they are disadvantaged in a manufactured environment created largely by and for right-handers. His account of this right-handed bias and its possible effects is more thorough and convincing than any I have read elsewhere, and effectively sets the scene for the final chapter, where he discusses practical ways in which the lot of the left-hander might be improved. I, a right-hander, am not entirely sure that left-handers will thank Coren, also a right-hander, for trying to organize them into an activist group, but most people, left- or right-handed, will be entertained, provoked and informed by this engaging book. □

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The triumph of disease?

Richard Davenport-Hines

Miasmas and Disease: Public Health and the Environment in the Pre-Industrial Age. By Carlo M. Cipolla. Yale University Press: 1992. Pp. 101. \$25, £16.95.

The Fourth Horseman: A Short History of Epidemics, Plagues and Other Scourges. By Andrew Nikiforuk. Fourth Estate: 1992. Pp. 200. £14.99.

TRIUMPHALISM has marred medical history throughout this century. For years, the subject was presented as an accompaniment to the March of Progress. Heroic scientists and humane physicians were depicted in an unceasing and unselfish battle against disease. The benefits of new therapies and vaccines were hailed: Nobel laureates such as Ehrlich were celebrated in Hollywood films, their discoveries taken as a sign that mortality could be postponed. Fifteen years ago, medical historians began to react against these simplifications. Physiological factors have lost their paramouncy. Scientific careerism is recognized. Both disease and the mediation of physicians have been increasingly analysed in the context of patients' social environment. This revisionism has brought many benefits, but can go miserably awry.

Revisionist techniques are exemplified at their best by the distinguished polymath Carlo Cipolla. His monograph is based on the records of the Florentine Health Board in the early seventeenth century, an epoch when Italy was at the forefront of European public health organization. He reproduces extracts from original medical reports that show the efforts of intelligent, inquisitive and eager physicians to fight disease in an unhygienic, superstitious culture. In addition, he provides interesting insights into the economic consequences of epidemics. Quarantine brought economic ruin to many communities, and when malaria was not fatal, its weakening of patients was a determining factor in economic stagnation and long-term poverty.

The rural masses of Tuscany were deterred from seeking medical treatment by poverty and because, as Cipolla writes, "physicians inspired feelings of reverent fear and peasants preferred to consult the local charlatan or wise woman". Given the failure of medical techniques, this preference probably saved peasant lives. As one physician reported in 1622, "more of those who are able to seek medical advice and treatment die than of the poor". Cipolla describes his story as "sad, unpleasant and often tragic". He writes with humanity, humility and scrupulous care, not

least of physicians whose knowledge now seems woefully limited.

By contrast, Andrew Nikiforuk's derivative and journalistic diatribe on epidemiology is arrogant, indignant and contemptuous. "One of the great lies of the twentieth century", he trumpets, is "that antibiotics, vaccines and doctors have saved us from pestilence." For the triumphalism of scientists conquering



Habit des Medecins, et autres personnes qui visitent les Pestiferes. Il est de marroquin de leuant, le masque a les yeux de cristal, et un long nez rempli de parfums

Dealing with the plague — a physician in protective clothing (from an eighteenth-century illustration).

microbes, he substitutes a hegemony of disease — what he calls "the golden age of pestilence". Bacteria are described as the planet's "eldest, brightest" life-form working together "as one big super-organism" and erupting in epidemics when humankind has "trampled its frontiers violating unwritten bacterial codes".

He seems more in sympathy with the 'superorganism' than with humans. His comments on his fellow beings vary from obtuseness to lofty cruelty. In the past, he writes in apparent admiration, "people didn't marry for sex or looks;

they carefully chose a partner for his or her ability to outlive an epidemic" — as if two millennia of erotic literature were a lie, and as if human emotions could be reduced to the eugenic equations beloved by twentieth-century totalitarianism.

In a key section he deprecates the more recent "obsessive desire to eradicate malaria" as "appalling" in its "racial, economic and political consequences". The elimination of malaria in Sri Lanka, for example, has led to overpopulation, which made civil war inevitable. "When people tinker with malaria", he moralizes, "they ultimately tinker with the structures of everyday life." If his argument has any meaning, it is that high mortality from malaria should be perpetuated in underdeveloped economies. Readers of Cipolla's account of the effects of malaria will recoil from the brutality of this.

Nikiforuk makes some fair points about the ecological destruction of the planet, and has legitimate criticisms of some medical practices, but his opposition to human meddling with "the total environment" is fanatical. Moreover, his ostensible concern with ecology frequently turns into righteous denigration of human choice — or "life-style" in the cant upon which he relies. "The passion for setting people right is in itself an afflictive disease", as Marianne Moore wrote. Nikiforuk's passion becomes pathological in his final chapter, a highly tendentious account of AIDS. He insists on false distinctions between the spread of the human immunodeficiency virus (HIV) in Africa and what he does not scruple to call the "gay plague" in the United States. Male homosexuality is "biological suicide", people with AIDS are "young middle-class men who thought they could defy biology" and HIV poses no threat to "white middle-class couples". Nikiforuk lingers in detail on sexual acts that he abhors, and becomes imaginatively overheated in places. Every paragraph of this chapter contains an assertion that could be contested, or a sentiment that repels.

The peculiarities of this unpleasant book go far beyond quaint eccentricity. It insults honest, if fallible, scientists and physicians. Patients are patronized and degraded. The chapters on malaria and HIV are a farrago of specious assertions and authoritarianism. Overall *The Fourth Horseman* is a discredit to environmentalism and a travesty of historical truth. □

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Warburg's wars

Walter Gratzer

Ein Genie Irrt Seltener . . . Otto Heinrich Warburg: Ein Lebensbild in Dokumenten. By Petra Werner. Akademie Verlag: 1991. Pp. 476. DM74.

A. J. BALFOUR avowed in old age that the chief lesson life had taught him was that nothing matters very much and hardly anything matters at all. The passing years brought no such consolation to Otto Warburg, for to him everything that touched his life and work mattered acutely until the end. His career of nearly 70 years was riven by disputes, pursued with a passion that was seldom slaked even by an adversary's death. That Warburg was withal a great man, a titan of modern biology, is not in doubt, for much of what is today taken for granted in biochemistry, cell biology and the analytical techniques on which they depend derives from his work.

The young Warburg grew up in a scientific hothouse presided over by his father, Emil, an illustrious physicist, intimate of Einstein and a friend of his son's first patron, Emil Fischer. Warburg revered Fischer, whose statue in bronze he caused to be erected half a century later outside his new research institute. Almost everyone else who crossed his path he regarded as fair game, starting in his twenties with the venerable Jacques Loeb, who met his vituperation with saintly forbearance. Warburg's career was already in full flower when the Kaiser's war intervened and he volunteered for the cavalry, in which he served for four years. Like J. B. S. Haldane, he seems to have been one of the few participants who thoroughly enjoyed the war, but where Haldane was described by his commander as the bravest but dirtiest officer in his unit, Warburg took especial pleasure in the martial glamour and the dashing uniform of his regiment of Uhlans. His mother, appalled at the lengthening odds against her son's survival in the cataclysm of 1918, appealed to Einstein to intercede for his release and eventually, but only shortly before the armistice, permission came for Warburg to return to his laboratory on the specious grounds that his work on photosynthetic algae might lead to a new food source for a starving populace.

Petra Werner has put together an absorbing commentary on Warburg's life and his social and scientific milieu in letters and documents. She has a telling quotation from a letter in which he extols the military virtues. The army taught him, he wrote, how to deal with

men and to obey commands — and by implication how to run his laboratory, where indeed orders were orders and no deviations from the director's code of conduct were countenanced. As a bachelor, with his own batman to look after him, Warburg had no sympathy with the demands of family life on his subordinates. Illness was no excuse for absence and death barely so: visits to the doctor, it was decreed, must wait until the annual vacation. Warburg's student, the future Nobel laureate Hans Krebs, was brusquely dismissed and advised to find himself another vocation, probably because he had remonstrated with the chief



Enjoying the war — Warburg served for four years in the cavalry.

about the abusive tone of his criticisms of some work by Keilin and Wieland.

Warburg was on poor terms with his fellow directors at the Kaiser-Wilhelm Institute; he regarded himself, generally no doubt with reason, as their intellectual superior and he made little attempt to conceal his opinion. Foreign visitors were, however, welcome in the laboratory and he was willing to learn from physicists and physical chemists, for he regarded science as indivisible and the barriers between disciplines as otiose. When he feared that he might be overextending the resources of his laboratory, he sought Walther Nernst's advice on whether he should concentrate on photosynthesis or on cancer; cancer, the guru pronounced, because photosynthesis after all works perfectly well. In the event he continued with both, and developed the oxidative theory of malignancy, to which he cleaved to the end of his life. His high-pressure oxygen therapy for

cancer was widely adopted in Germany and the enthusiasm for it, whipped up by the press, made him a public figure. It was not the only nostrum to which he lent his name. The waters of the European spas were still widely held to have all but miraculous curative properties, probably in the main because of the consuming Central European preoccupation with bowel motions. (It used to be said that Karlsbad was built on undelivered faeces.) Warburg's association with the pharmaceutical company Schering led to the marketing, with his endorsement, of tablets that would generate in the glass either Karlsbad or Marienbad water. The uninhibited advertising literature, bearing Warburg's name and fulsome testimonials from satisfied customers, makes curious reading.

Success was not enough for Warburg: his rivals had to be seen to fail. The Nobel prize for Wieland enraged him. The prize, he wrote to his sister, signified nothing, awarded as it was by no more than a gang of drunken Swedes. Petra Werner documents the celebrated episode of high comedy when *The Times*, mistaking Warburg for his namesake and distant relative, a professor of botany at the University of Jerusalem, printed an obituary, also taken up by *Nature*. The piece failed, in Warburg's view, to do his achievements justice and omitted even to credit him with the discovery of the "old yellow enzyme" of intermediary metabolism. He is believed to have written a strongly worded letter to *The Times*.

The rise of the Nazi party caused a swift exodus of scientists from Germany. Warburg regarded flight as undignified and stayed put. At first he offered at least passive resistance to the depredations of the regime, but as dissent grew more dangerous he became increasingly concerned to protect his position. He had never acknowledged his Jewish ancestry, and now demurred when his sister begged for help for her husband, the distinguished chemist von Wartenberg, who had been dismissed for having a half-Jewish wife. Warburg now found that upright Germans were increasingly avoiding his company. Meyerhof, whom he respected, was denounced by the odious Richard Kuhn (then an institute director, and still one after the war) for harbouring non-Aryan research assistants, and left for the United States. Shortly thereafter, Warburg was relieved of his position, but a little later he was reinstated. Werner gives reasons for believing that he owed his survival to the conviction of highly placed patrons that he would (as he had himself repeatedly claimed) shortly find a cure for cancer and thus shed glory on German science as well as promoting their own longevity;

Hitler, moreover, was known to have a morbid fear of cancer and may himself have sanctioned Warburg's reclassification from half-Jewish to quarter-Jewish to circumvent the race laws. At all events, he spent a tranquil war, pursuing his researches, and was permitted even to maintain his stable of thoroughbreds.

In 1945 the Russians arrived and Warburg levanted to his country house on the island of Rügen, leaving the surviving members of his staff to protect the institute as best they could against the rapine of the occupiers. This act of abnegation caused much rancour. One of the most absorbing passages of the book is a long extract from the diary of Warburg's assistant, Lüttgens, chronicling the life of the institute during this tumultuous period. In the end the contents of the laboratory were packed into crates and shipped off to Russia. Left without the means to carry on experimental science, Warburg dedicated himself to writing two monographs, full of invective against his scientific rivals. There followed a disastrous visit to the United States at the invitation of Robert Emerson, who hoped that the issue of the quantum yield of photosynthesis, which had become a running sore, could be cleared up by collaborative experimental work in Chicago. Warburg could not bring himself to concede defeat and left in a huff. The death of his old adversary James Franck did not appease his wrath; he took exception to a generous obituary and wrote an intemperate rejoinder in a journal. In a letter to Krebs, Max Delbrück expresses his disgust at the manner in which Warburg conducted his polemics and owned that this alone had deterred him from entering the field.

In 1953, Warburg at last took possession of a fine new research institute that the Max-Planck Society had built for him in Dahlem. He was then 70 and was to continue working for another 17 years and 240 papers, up to two days before his death, which was greeted with thunderous eulogies.

Petra Werner has skilfully resurrected the scaly old basilisk for her readers and illuminated a richly interesting period of scientific history. Her book admirably complements Krebs's biographical memoir of 11 years ago (*Otto Warburg: Cell Physiologist, Biochemist and Eccentric*). There is a well-chosen collection of photographs. The title — an assertion that is not perhaps borne out by the circumstances of Warburg's life (or by that of many other geniuses) — comes from a tribute by M. L. Anson, Warburg's one loyal American admirer. □

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The politics of our genes

W. F. Bynum

Eugenics, Human Genetics and Human Failings: The Eugenics Society, its Sources and its Critics in Britain. By Pauline M. H. Mazumdar. *Routledge: 1992. Pp. 373. £40, \$74.50.*

It is an historical commonplace that the eugenics movements of half a century ago were fuelled by race in Germany and the United States, and by class in Britain. American eugenicists were concerned with immigration, especially from Asia and Eastern Europe; German eugenicists with the presence of Jews and other 'non-aryans' in their midst; and the British with their perceived class-related differences in fertility. After the atrocities of the Nazis were exposed, eugenics acquired a dirty name, and post-war science and social policy played down hereditary determinants in favour of social, cultural and environmental ones. And eugenics remains as a salutary tale of ideology and intolerance masquerading as science; repression pretending to be progress.

Like many commonplaces, this one contains the virtue of first-order explanatory power, but little more. As Pauline Mazumdar's study of Britain's Eugenics Society makes clear, there was never a complete consensus among the society's members, either in terms of the 'facts' of human inheritance or the consequences for human betterment to be drawn from them. Nor, in her account, was the revulsion caused by the holocaust a major reason why the Eugenics Society was not the force in the 1950s that it had been a couple of decades earlier. Rather, she attributes this decline largely to the changed social climate of the early years of the Welfare State, and to the sustained criticism of eugenic methods and assumptions by a number of British scientists, mostly of left-wing politics, and including Lancelot Hogben, J. B. S. Haldane (the centenary of whose birth falls this year) and Lionel Penrose. In particular, the favourite method of the eugenicists — the collecting and evaluation of elaborate human pedigrees — was discredited and replaced by the more sophisticated tools of factor analysis and chromosome linkage. These, Hogben insisted, were ethically neutral and could yield a sound science of human heredity. Ironically, they derived much inspiration from German mathematical models — *Vererbungsmathematik* — and their application to the analysis of human blood groups.

Mazumdar offers a shrewd and largely

convincing interpretation of the interplay of politics and science in the work of Hogben, Haldane and Penrose, even if she never actually confronts the issue of why human genetics after the Second World War might be thought to be more value-free than the eugenics it replaced. Insofar as there was an *experimentum crucis*, it was probably Penrose's survey of 1,280 cases of mental subnormality, carried out in the 1930s at the Royal Eastern Counties Institution at Colchester. His brief was to examine patients who had been diagnosed as mentally defective and to determine the relative roles of heredity and other factors in the aetiology of their condition. Apart from those with the specific conditions of Down's syndrome and phenylketonuria, he found it impossible to categorize most of the patients with precision, and they had rather boring and unrevealing family histories.

Studies such as Penrose's did much to undermine eugenic claims that pedigree studies unambiguously demonstrated the hereditary nature of many undesirable social traits, and the preponderance of these traits in the social class that was often called the residuum. Nevertheless, Mazumdar correctly emphasizes that any crude polarization of students of human heredity in the 1930s into eugenic and anti-eugenic camps is misleading. The longtime secretary of the Eugenics Society, Carlos P. Blacker (inexplicably rechristened 'Charles' by Mazumdar) managed to remain on good terms with many who were critical of the society's aims and methods, publishing one of Hogben's papers in a volume he edited, and trying to adapt the society's policies to the political realities of Britain after the Second World War. More generally, eugenicists and their critics often came from similar backgrounds, sat on many of the same committees, saw each other socially and read each other's books and papers. The centre of gravity of much of Mazumdar's book is the senior common room at University College London, where Karl Pearson, Cyril Burt (dubbed 'Cecil' in the index of this book), Haldane and Penrose taught.

This institutional context of eugenics and its critics was presented rather more explicitly in Daniel J. Kevles' *In the Name of Eugenics* (Knopf, New York 1985; Penguin, London, 1986), and readers of that splendid monograph will find much in Mazumdar's study that is already familiar. They will also recognize that Mazumdar's rather stolid style is a far cry from the lightness of Kevles' erudition. Nevertheless, Mazumdar has not simply been treading in Kevles' footsteps. She offers some new insights into the background of the Eugenics Society, the influence of German mathematics on British geneticists and the changing offi-

cial preoccupations of the Eugenics Society, particularly in relation to the nature of and possible remedies for mental disorder and mental subnormality. One would hardly trade this volume for that of Kevles; but neither should we discount the value of Mazumdar's own contributions to a theme still pregnant for us, the generation of the genome. □

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■ In *The Hour of Eugenics: Race, Gender, and Nation in Latin America*, Nancy Leys Stepan compares the eugenics movements in Mexico, Brazil and Argentina with those of Britain, the United States and Germany. Published by Cornell University Press, price \$31.50.

Mere platonic invention?

Colin Renfrew

The Flood from Heaven: Deciphering the Atlantis Legend. By Eberhard Zangger. Sidgwick and Jackson: 1992. Pp. 256. £17.50, \$22. (To be published in the United States by William Morrow on 20 August.)

PLATO has a lot to answer for. In setting out the story of the vanished civilization of Atlantis (for which his writings are the only source), he has been the root cause of a great deal of unprofitable speculation. Some of the speculation has been fanciful beyond the bounds of reason, whereas other versions, such as that presented in this book, are honest attempts to make some sense of what he wrote. The story as reported by Plato (allegedly told by priests in Egypt to the Greek sage Solon some centuries earlier) places the doomed civilization in the Atlantic Ocean, and its demise some 9,000 years before Plato. In the light of current understanding, neither of these claims can be correct, and all modern authorities who take the story seriously are obliged to alter both the location and the date. That is where the trouble begins.

In recent years, a favourite contender has been the volcanic island of Thera in the Cyclades. For here, at least, was a worthy cataclysm, resulting in the destruction and burial of the Bronze Age city somewhere around the seventeenth century BC at what is now Akrotiri. Several authors have claimed that Thera fits the bill — as it may well do if place, time and other details are conveniently varied. For a while, the entire civilization of Minoan Crete was seen as a candidate, the collapse of the Minoan



Mary Evans

Digging for Atlantis? Excavation of Hissarlik, claimed as Troy, by H. Schliemann in 1873.

palaces being plausibly attributed to the Thera eruption. But that suggestion no longer finds favour, and another Atlantis theory vanishes beneath the waters.

Eberhard Zangger has an alternative and ingenious candidate: for him Atlantis equals Troy. As a geomorphologist, he is well placed to argue that the ancient topography of the site of Hissarlik, excavated and claimed as Troy by Heinrich Schliemann, has changed significantly since the time of the Trojan War, some 3,000 years ago. His suggestion has one notable strong point, to my mind. For in Plato's Atlantis story it is stated that the forces of Solon's homeland (Athens) had defeated those of Atlantis before the cataclysm that brought about its disappearance. And of course the most important feature of the Greek legends relating to Troy was the Trojan War itself, and the capture of the city by the Hellenic forces.

Inevitably, however, Zangger has to modify the Platonic data on time and place. Moreover, he accepts both the real existence of the Trojan War (a point disputed by many scholars, most notably the late Sir Moses Finley) and its identification with the site of Schliemann's excavations. These are inevitable difficulties. They are compounded by the special arguments needed to make the Trojan landscape conform to the Platonic description of Atlantis, and by the absence of any satisfactory natural calamity to bring about the disappearance of Troy. For, if correctly identified, the site was still there in classical Greek times, when a temple was built, and remained to await the spade of Schliemann.

Zangger's own recent work has been at the Mycenaean citadel of Tiryns in the

Argolid (southern Greece). There he has found good evidence of a large flood, probably earthquake-induced, that covered much of the lower city with mud around 1200 BC. But although much is made of this circumstance in his book, he does not imply a larger, pan-Aegean catastrophe that might have engulfed Troy at that time (and, as noted above, the site of Hissarlik itself remains obstinately un-engulfed, although the topography, and especially the coastline, have certainly changed since then).

This book is well written. The relevant texts from Plato are printed in full and carefully discussed. The case that Zangger is making is not overstated. In one sense it is perhaps understated, because the relevance of his own observations at Tiryns to the history of Troy is not spelt out very clearly, and indeed remains to me obscure. For the sceptic, therefore, for whom even the historical reality of Troy and the identification of Hissarlik with it are problematic, the central thesis that Atlantis was Troy is unpersuasive. As Atlantis books go, this is a perfectly sane and well-argued one, written by a competent scholar. But Aristotle was one of the first to suggest the view, pithily expressed by Francis Cornford in 1937 (and quoted by Zangger), that "serious scholars now agree that Atlantis probably owed its existence entirely to Plato's imagination". Until Egyptologists unearth the hieroglyphic inscriptions upon which Solon's Egyptian informants supposedly relied, I shall incline towards Aristotle and Cornford rather than towards Plato and Zangger. □

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